THE
BOTANICAL GAZETTE

CHARLES E. OLMSTED · *EDITOR*

VOLUME 108



THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

CAMBRIDGE UNIVERSITY PRESS, LONDON

PUBLISHED
SEPTEMBER, DECEMBER, 1946, AND MARCH, JUNE, 1947

COMPOSED AND PRINTED BY THE UNIVERSITY OF CHICAGO
PRESS, CHICAGO, ILLINOIS, U.S.A.

TABLE OF CONTENTS

		PAGE
Action spectrum for the photoperiodic control of floral initiation of short-day plants	*M. W. Parker, S. B. Hendricks, H. A. Borthwick, and N. J. Scully*	1– 26
Breeding behavior and chromosome numbers in progenies from twin and triplet plants of *Poa pratensis*	*Etlar L. Nielsen*	26– 40
The origin of multiple macrogametophytes in *Poa pratensis*	*Etlar L. Nielsen*	41– 50
On *Cardiocarpon* and some associated plant fragments from Iowa coal fields	*Fredda D. Reed*	51– 64
Control of flower formation and fruit size in the pineapple	*J. van Overbeek*	64– 73
A comparison of histological responses of bean plants to tryptophane and to low concentrations of indoleacetic acid	*Mary Aileen Murray and A. Geraldine Whiting*	74–100
Responses of *Pteris longifolia* to applications of ammonium 2,4-dichlorophenoxyacetate	*Bernard Strickler*	101–114
The effect of indoleacetic acid upon initiation and development of hypocotyledonary bud primordia in flax	*George K. K. Link and Virginia Eggers*	114–129
Megasporogenesis and development of the embryo sac of *Allium cernuum*	*John B. Murphy*	129–136
Flower-bud formation and development in *Mangifera indica*	*Margaret J. Mustard and S. J. Lynch*	136–140
Variation in ecotypes of *Poa pratensis*	*D. C. Smith, E. L. Nielsen, and H. L. Ahlgren*	143–166
Reactions of decapitated bean plants to certain of the substituted phenoxy compounds	*J. M. Beal*	166–186
Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching	*Robert W. Hanks*	186–191
Histological responses of bean plants to nicotine and to wounding	*A. Geraldine Whiting and Mary Aileen Murray*	192–219
Effects of pre-storage treatment of hardwood and pine seedlings with α-naphthaleneacetic acid	*Roger D. Way and T. E. Maki*	219–232
Volumetric studies of seeds, endosperms, and embryos in *Pinus ponderosa* during embryonic differentiation	*John T. Buchholz*	232–244
A cytological study of *Uromyces aloës*	*M. J. Thirumalachar*	245–254
Characteristics of the nitrogen-fixing enzyme system in *Nostoc muscorum*	*R. H. Burris and P. W. Wilson*	254–262
Cytological studies in the Alismaceae	*Walter V. Brown*	262–267
The role of organic matter, especially manure, in the nutrition of rice	*James Bonner*	267–279
Changes which occur in apple tissue upon treatment with various agents and their relation to the natural mechanism of softening during maturation	*Jean H. Griffin and Z. I. Kertesz*	279–285
Preliminary results with the use of 2,4-dichlorophenoxyacetic acid as a spray-oil amendment	*William S. Stewart and Walter Ebeling*	286–294
Analysis of levulins, inulin, and monosaccharides in guayule	*Hamilton P. Traub and M. C. Slattery*	295–299
Growth-regulating substances as herbicides	*E. J. Kraus and J. W. Mitchell*	301–350
The comparative morphology of the Fagaceae. I. The genus *Nothofagus*	*LaDema Mary Langdon*	350–371

		PAGE
Morphology of spore forms and heteroecism of the giant bamboo rust, *Dasturella divina*	*M. J. Thirumalachar, M. J. Narasimhan, and K. S. Gopalakrishnan*	371–379
Growth and morphology of tobacco tissue cultures *in vitro*	*Samuel M. Caplin*	379–393
Development of ovule and megagametophyte in pomegranate	*J. R. King*	394–398
Ether-soluble organic acids and buffer properties of citrus peels	*Walton B. Sinclair and Desire M. Eny*	398–407
Survey of higher plants for presence of anti-bacterial substances	*Lesley E. Hayes*	408–414
Selective herbicidal effects of 2,4-dichlorophenoxyacetic acid applied to turf in dry mixtures with fertilizer	*Paul C. Marth and John W. Mitchell*	414–420
Response of potato plants to spray applications of certain growth-regulating substances	*Ora Smith, M. A. Baeza, and J. H. Ellison*	421–431
Growth of field crops in soil treated with chemical growth-regulators	*D. L. Taylor*	432–445
Spectrophotometric method for determination of 2,4-dichlorophenoxyacetic acid	*Robert S. Bandurski*	446–449
On the life-history of *Vanilla planifolia*	*B. G. L. Swamy*	449–456
Anatomy of Washington navel orange rind in relation to water spot	*Flora Murray Scott and Katharine C. Baker*	459–475
Citrus leaf stomata: structure, composition, and pore size in relation to penetration of liquids	*F. M. Turrell*	476–483
Pigment glands of cottonseed. II. Nature and properties of gland walls	*Charlotte H. Boatner, Catherine M. Hall, Mary L. Rollins, and Leah E. Castillon*	484–494
Photomicrographic comparison of milkweed bast and cotton lint in relation to cell-wall structure of other natural cellulose fibers	*Mary L. Rollins*	495–510
Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime	*Olive H. Kries*	510–525
Some results of cross-pollination on *Lilium regale*	*J. M. Beal*	526–530
Developmental sequence of embryo and endosperm in apomictic and sexual forms of *Poa pratensis*	*Ellar L. Nielsen*	531–534
Response of guayule to alternating periods of low and high moisture stresses	*H. M. Benedict, W. L. McRary, and M. C. Slattery*	535–549
Effects of fruit thinning on almond fruits and seeds	*George A. Baker and Reid M. Brooks*	550–556
Effect of duration and intensity of light upon flowering in several varieties and hybrids of castor bean	*N. J. Scully and W. E. Domingo*	556–570
Influence of potassium and sodium on metabolism of peanut cotyledons during germination	*Hubert J. Dyer*	570–581
Development of the embryo of *Thymelaea arvensis*	*J. Venkateswarlu*	581–586
CURRENT LITERATURE		141, 299, 457, 587

For titles of books reviewed see Index under author's name

ERRATA

Volume 108

P. 296, sixth line from bottom: *for* inulin *read* levulins

P. 298, eleventh line from top: *after the word* levulins *add* plus inulin

ACTION SPECTRUM FOR THE PHOTOPERIODIC CONTROL OF FLORAL INITIATION OF SHORT-DAY PLANTS

M. W. PARKER,[1] S. B. HENDRICKS,[2] H. A. BORTHWICK,[3] AND N. J. SCULLY[4]

Introduction

It has been shown that floral initiation in short-day plants can be prevented by brief periods of illumination applied near the middle of the dark period (15). The interruption, depending upon the light intensity, need not be more than a few seconds. A study of the effectiveness of different wave lengths of light on this reaction has been made, and curves relating wave-length to effectiveness, which may be referred to as the action spectrum, have been constructed. Only by means of quantitative data which can be expressed in the form of an action spectrum curve can information be obtained as to the fundamental nature of the photoreactions that are responsible for the photoperiodic responses of plants. Many investigators, all using filtered radiation, have studied the influence of various regions of the spectrum on this reaction, but none has made quantitative determinations of the relative effectiveness of the different wave-length bands.

Four conditions were established in experiments for determining the action spectrum. First, use was made of the inhibiting action on floral induction of brief periods of irradiation applied at or near the middle of the dark period. Second, the radiation applied at these times consisted of relatively narrow wave-length bands of high spectral purity. Third, the source of radiant energy for these dark-period interruptions was of high intensity in all regions of the visible spectrum. Fourth, the biological response investigated was restricted to floral initiation.

[1] Physiologist; [2] Senior Chemist; [3] Senior Botanist; [4] Associate Physiologist; Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Plant Industry Station, Beltsville, Md.

Review of literature

RAZUMOV (16), working with both long-day and short-day plants, concluded that radiation from the red and yellow parts of the spectrum, when used to extend short natural photoperiods, was equivalent to daylight in its effect on the photoperiodic reaction, whereas green, blue, or violet radiation was equivalent to darkness. Both KATUNSKIJ (10) and KLESHNIN (11), on the contrary, found that the photoperiodic reaction could be influenced by radiation from any part of the visible spectrum but that the intensities required differed from one region to another. KATUNSKIJ reported that red was most effective, with yellow-orange a close second and blue third in effectiveness. He found green to be the least effective region and noted that there was a close similarity between photoperiodism and photosynthesis in their dependency on the spectral composition of light. KLESHNIN called special attention to the fact that it was necessary to use low intensities of radiation if differences in effectiveness of different spectral regions were to be distinguished, and he suggested that failure of some workers to consider this fact probably accounted in part for contradictory conclusions.

WITHROW and BENEDICT (18) investigated the effects of several different wavelength bands and found that orange and red resulted in earliest blooming and greatest number of flowers with pansy, stock, and aster. Blue, green, and yellow radiation had little effect on pansy and stock, but with aster all wave-length bands were effective in hastening flowering. WITHROW and BIEBEL (19) extended these investigations to include short-day plants and found that the red wavelength band, which was most effective in promoting flowering of long-day plants, was likewise most effective in preventing flowering of short-day plants. WITHROW and WITHROW (20) carried out further work with red radiation and studied the effects of blue light in greater detail. They were aware that small energies of red radiation were sufficient to produce the effects observed and were not certain that in previous experiements these levels had not been exceeded by extraneous unfiltered light. Their results showed almost no effect of blue radiation and confirmed previous observations as to the effectiveness of the red region. They concluded that the leaf pigment controlling the photoperiodic response of all plants with which they worked absorbs strongly in the red, less so in the yellow-green, and least in the blue regions of the spectrum.

Several experimental conditions could have contributed to the diverse results obtained with blue radiation. First, these studies were made with filtered radiation, and the filters used by the different workers were not identical. In most cases they transmitted very wide wave bands, and some authors appear to have attached insufficient importance to the weak transmission that may have occurred in wave bands outside the principal region of transmission. Second, most workers failed to employ energy levels near the threshold required in the different wave bands to produce the photoperiodic effect. The energy in the red was frequently higher than necessary and in the blue was too low to produce an effect. Third, the biological response measured could have been more sharply defined in many cases. Basically, all workers were concerned primarily with the spectral conditions that permitted transition of the plants from a purely vegetative development to a reproductive one. The effectiveness of a given treatment was in nearly all cases judged by the ultimate formation or lack of formation of macroscopic flower buds or open flowers. This procedure required that experimental treatments be of several weeks' duration. It assumed that the plants had not initiated flower primordia before the treatments were applied and further assumed that the effectiveness of a given treatment in causing floral initiation was similar to its effectiveness in promoting the subsequent growth and development of these flower primordia into macroscopic buds and flowers. This assumption is not valid in the case of soybean, and probably many other plants would have similar behavior. Soybean plants with established flower primordia can be given photoperiods that will prevent their development into flowers or fruit.

Biological material and experimental procedures

The short-day plants used in this investigation were soybean, *Soja max* (L.) Piper var. Biloxi, and cocklebur, *Xanthium saccharatum* Wallr. The plants were grown in soil in $3\frac{1}{2}$- or 4-inch pots in the greenhouse and were produced in sufficient number so that very uniform populations could be selected for each experiment.

All experiments with Biloxi soybean by this project for the past several years have been conducted with seed derived from the same original source, new crops of which have been produced from year to year. This strain produces very uniform plants and contains no types that vary significantly from the rest in their photoperiodic behavior. Most of the cocklebur seed was obtained along a road near the grounds of the Bureau of Plant Industry Station. For preliminary experiments, however, seed was obtained through the courtesy of Dr. E. J. KRAUS from the source used by HAMNER and BONNER (7) and referred to by them as *X. pennsylvanicum*. The photoperiodic responses of the plants from the two sources were identical, and both were determined by Dr. S. F. BLAKE as *X. saccharatum* Wallr.

Because of the limited amount of leaf area that could be irradiated with the spectrograph, it was necessary to reduce the leaf surface by defoliation as much as was possible without interference with the capability of the plant to respond to photoperiodic treatment. With soybean all expanded foliar organs except the terminal leaflets of the third compound leaf were detached. It had been found in earlier studies (3) that initiation of flower primordia by plants defoliated in this way was not impaired. With cocklebur all leaves but the most recently fully expanded one on each plant were removed. The leaf irradiated was usually located at the sixth or seventh node.

For all experiments the plants were grown under conditions of long photoperiod until they were ready for treatment. These photoperiods consisted of all the natural light available at the season the plants were growing plus approximately 40 foot-candles of incandescent-filament radiation from sundown to 2:00 A.M. The photoperiods thus exceeded 18 hours in the winter and 20 or more hours in the summer. This procedure has always been found to prevent floral initiation of Biloxi soybean and cocklebur, as determined by dissections made of representative plants at the time of the experiments. The experimental lots of plants were selected and moved from the greenhouse into the controlled-environment rooms described by PARKER (14). Here the soybeans were subjected 6 days, and the cocklebur 3 days, to short photoperiods using the methods described in detail below. During these experimental periods the plants differentiated a few new structures and made a small amount of growth but not enough to permit final determination of the effects of the treatment, so it was necessary to allow a few more days for their further development before the stem terminals were dissected. During this period the plants were again held in the greenhouse on long days. Dissections were made of all soybeans 14 days, and of all cocklebur 10 days, after the beginning of the experimental treatment.

Use of the growth chambers assured constant growing conditions during the experimental periods. Light intensities in the greenhouse both before and after the experimental treatments were subject to natural fluctuations, but, in general, these differences were not found to interfere with the results except during periods of unusually dark weather. At such times the plants were held on long photoperiods in the growth chambers for a few days prior to and following treatment.

During the experimental period the soybeans received 10-hour, and the cocklebur 12-hour, photoperiods of carbon-arc plus incandescent-filament radiation having a combined intensity at the

leaf surface of about 2000 foot-candles. Treatment of soybean and cocklebur in these growth chambers with the respective lengths and numbers of photoperiods indicated above invariably resulted in the production of flower primordia if the dark periods were uninterrupted. Floral initiation could be completely inhibited, however, if the dark periods were interrupted near the middle by brief periods of illumination of sufficient energy. It was thus possible through the application of a range of levels of energy in the form of dark-period interruptions to determine the threshold amount of energy that would just prevent flowering. These interruptions were made with filtered or white light in the reciprocity experiments and with relatively narrow wave-length bands of light from the spectrograph in all the action spectrum experiments. The energy levels required were such that, with the light intensities available, the duration of interruption of the dark periods ranged from about 1 to 25 minutes, except in a few experiments concerned primarily with the blue-violet end of the spectrum, in which interruptions as long as 40 minutes were necessary to inhibit floral initiation. In most experiments five different energy levels were used at each wave-length band tested. The sum of these five different periods of interruption was usually less than 1 hour, so that it was possible to complete all the interruptions in duplicate within a 2-hour period beginning 30 minutes before and ending 90 minutes after the middle of the dark period. This procedure, repeated in a second growth chamber operating on a cycle 2 hours later than the first, increased the number of replications of the entire experiment to four. In an average experiment fourteen or more bands of the spectrum were investigated in quadruplicate at five energy levels. The total number of plants involved in such experiments was 280 or more, exclusive of the controls which received short-day treatments but no dark-period interruptions. The individual lots of plants were brought from the control rooms and with the aid of a blue-violet safe-light, described below, the leaflets were placed in their respective positions on a board located in the image plane of the spectrograph.

The experimental results for soybean are expressed as total nodes at which flower primordia were formed and for cocklebur as total plants forming flower primordia. In soybean evidence for the effectiveness of a given treatment with short photoperiod is found in the production of flower primordia in the axil of the leaf situated several nodes below the terminal on the main axis (2). If the flower-forming stimulus is relatively weak, flower primordia may be formed only at this one node; but if the stimulus is stronger, they may be formed at one or more additional nodes above. The number of nodes on the main axis at which flower primordia occur is thus a semiquantitative measure of the effectiveness of the treatment. These values rather than the number of plants having flower buds are thus presented for soybean. Only when the stimulus is very strong and applied for a prolonged period does the terminal meristem give rise to a terminal inflorescence. In none of these experiments, however, was the treatment of sufficient intensity and duration to result in such a response.

In cocklebur, on the other hand, an effective treatment with short photoperiod results in the initiation of a terminal inflorescence. The only obvious difference in behavior of cocklebur plants that have received different intensities of photoperiodic stimulation is in rate of de-

velopment of the inflorescences. These differences can be expressed only by assigning the plants to various categories on the basis of size of the terminal inflorescences. This is unsatisfactory because of the possible influence of other factors on their rate of growth. For this reason the only record taken for cocklebur was the presence or absence of a recognizable inflorescence primordium, regardless of size.

Spectrograph methods and apparatus

Experiments designed to give the action spectrum for photoperiodic response required use of radiation of high intensity in a restricted wave-length region over an area somewhat larger than a leaflet of soybean or leaf of cocklebur. The wavelength band over a particular leaf had to be as narrow as possible, consistent with supplying energy adequate to suppress floral initiation as a result of exposures of less than an hour even in the least effective portions of the visible spectrum. This was achieved by means of the spectrograph described in the following paragraphs. Other experiments, performed to test the validity of the procedure used with the spectrograph, required only incandescent-filament lamps with or without filters.

The optical path in the spectrograph is shown in figure 1. Optical elements were a 25-cm. front-aluminized spherical concave mirror having a focal length of 2 meters, and two equilateral 60° flint glass prisms, with 14-cm. faces and 16.3 cm. in height. The slit and the focal plane were at the respective foci of the spherical mirror, thus giving a convergent beam through the prisms. The convergence leads to a loss of resolving power and thereby of spectral purity, which, however, is far within the limits required by the various experiments. The prisms were set at minimum deviation for the mercury green line. Light passing through the prisms was reflected from a front-aluminized flat mirror which was mounted so that it could be rotated about an axis in the mirror surface. Rotation was effected by means of a tangent screw on a 60-cm. arm. By turning the flat mirror, a given wave length of radiation could be brought to a specific position in the image plane of the spherical mirror. The image plane in most of the experiments was about 14 meters from the spherical mirror, which was adjustable in position to provide for focusing at any given position. The instrument and the light source, external views of which are given in figures 2*A* and 2*B*, were housed in a light-tight room having a length of about 18 meters.

Baffles were placed in the spectrograph to trap scattered light. Scattered light within the spectrum in the focal plane of the spherical mirror was reduced to an exceedingly low value, less than 0.1%, by the great distance from the last scattering surface, namely, that of the flat mirror. Constancy of light intensity throughout the course of an experiment was monitored with a light meter, having a barrier-layer type of photocell, placed in a portion of the spectrum from the edge of the first prism.

The slit of the spectrograph was 1.2 cm. high and could be adjusted to give widths as great as 1.5 cm. It essentially acted as a diaphragm on the projected image of the positive crater of the carbon arc which was used as a light source in most of the experiments. The carbon arc[5] was of the rotating cathode type using $\frac{9}{16}$-inch cored positive carbons. It could be operated at 12 kw. power input, and

[5] Supplied through the courtesy of Warner Brothers Theatres.

after several minutes' burning the light intensity at the monitor remained constant to within 3%. Under such operation a carbon lasted about 1 hour, and, although the arc would operate without attention for periods of 10–20 minutes, it was checked much more frequently to avoid deviations in energy output. Projection bulbs were used as light sources in some of the experiments with the red portion of the spectrum.

An adjustable slit that could be placed in the image plane of the spherical mirror served to define the spectrum for energy and wave-length calibration. The radiation passing this slit was focused on the receiver of a 10-junction thermopile, the response of which was calibrated with the aid of a standard lamp placed 6 meters from the slit. Wave-length calibration was carried out with the spectrograph slit illuminated by a low-pressure mercury arc. The spectrum position during the course of an experiment was checked against the absorption bands of a Didymium glass filter (Corning no. 512).

The dispersion of the spectrograph at 5000 A. is 15 A. per cm. At this dispersion the radiant intensity at 5000 A. can be as great as 3000 ergs per sq. cm. per sec. for an effective slit width of 100 A. In some experiments the distance to the image plane was shortened by moving the spherical mirror toward the slit, thereby decreasing the dispersion, in order to double the energy incident on a leaf in a given time. The spectrum for the greater image distance, which was generally used, measured about 2 meters over the visible region (4000–7000 A.) and had a height of about 10 cm.

Stations upon which leaflets were placed during irradiation were established on a board set in the image plane, as shown in figure 3*A*. The position of the spectrum was fixed with reference to a particular station in a given experiment. In all experiments with wave-length bands below 5500 A.—i.e., in the violet, blue, and green—leaflets were placed flat on the board and thus normal to the incident light beam (fig. 3*A*). A single leaflet was restricted to 10 cm. of the spectrum and subtended about 50 A. at 4000 A. and 150 A. at 5000 A. At the highest intensities an individual station thus covered a spectral region approximately one and one-half times the effective slit width.

In the region of the spectrum above 5500 A. dispersion decreased to such an extent that a single leaflet placed flat would have subtended about 200 A. in the region of 6000 A. Since use of such a

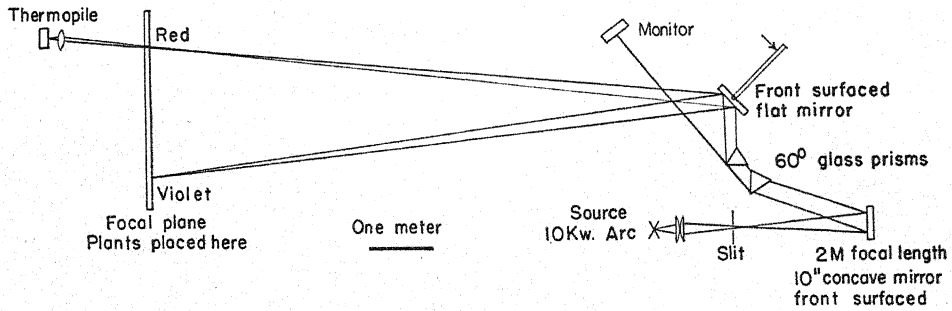


FIG. 1.—Diagram of optical path in spectrograph used for irradiation of plant leaves

wide band was objectionable, the leaflets for most experiments in this region were held on wedges at an angle of 30° to the light beam, as shown in figure 3*B*. The leaflets thus intercepted bands of only half the previous width or about 75 A. at 5500 A. and 100 A. at 6000 A. The purity of the spectrum in this region was increased by decreasing the slit width. The consequent reduction in energy was not objectionable because lower energies in this region of the spectrum were still effective.

Another procedure in a few experiments consisted in use of a lens arrangement that spread a small wave band of the red over a large area in which leaflets could be placed normal to the beam. A lens of 20-cm. focal length was mounted behind a slit in the image plane of the

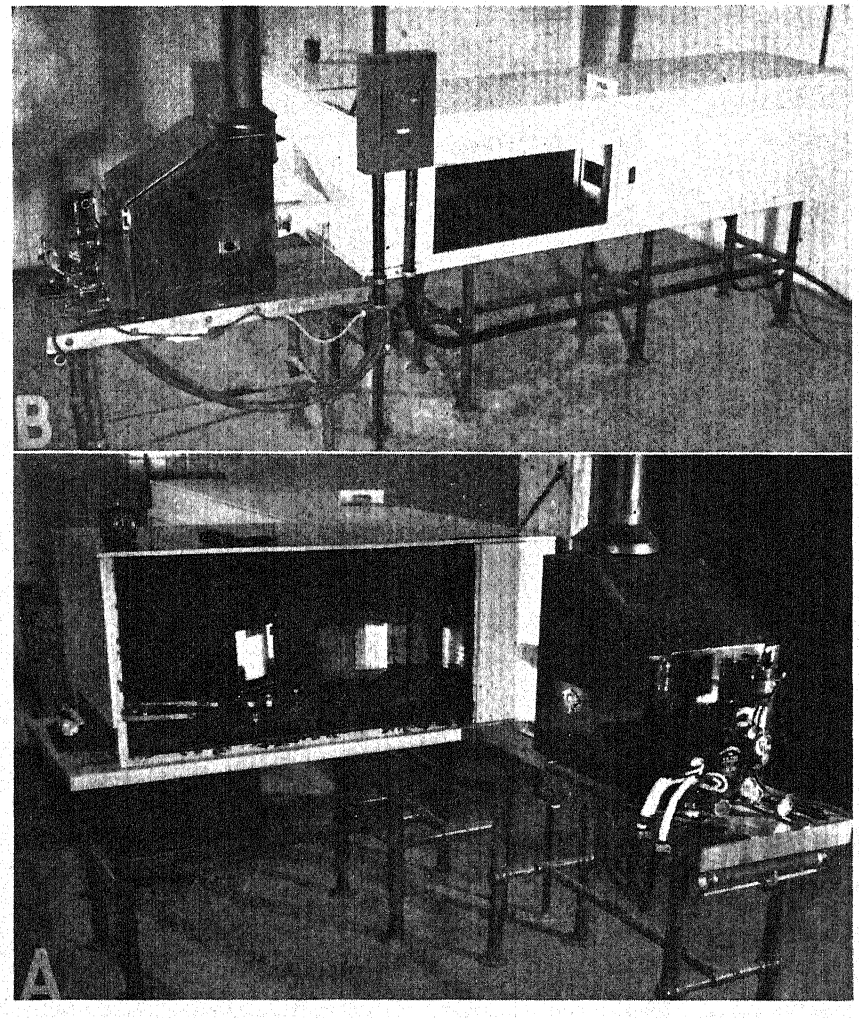


FIG. 2.—Views of spectrograph. *A*, front view with panel removed, showing two 60° flint-glass prisms and front-aluminized flat mirror attached to tangent screw. *B*, side view, showing light beam after passing slit located in cone attached to front of arc lamp.

spectrograph upon which a portion of the red end of the spectrum was incident. The intensity at the surface of the leaflets was dependent upon their distance from the lens and could be so adjusted that the exposure times could be increased to the same order as those in the blue. This arrangement also permitted direct comparison of the effectiveness of radiation at the two ends of the spectrum with the leaves placed normal to the incident beam in both cases.

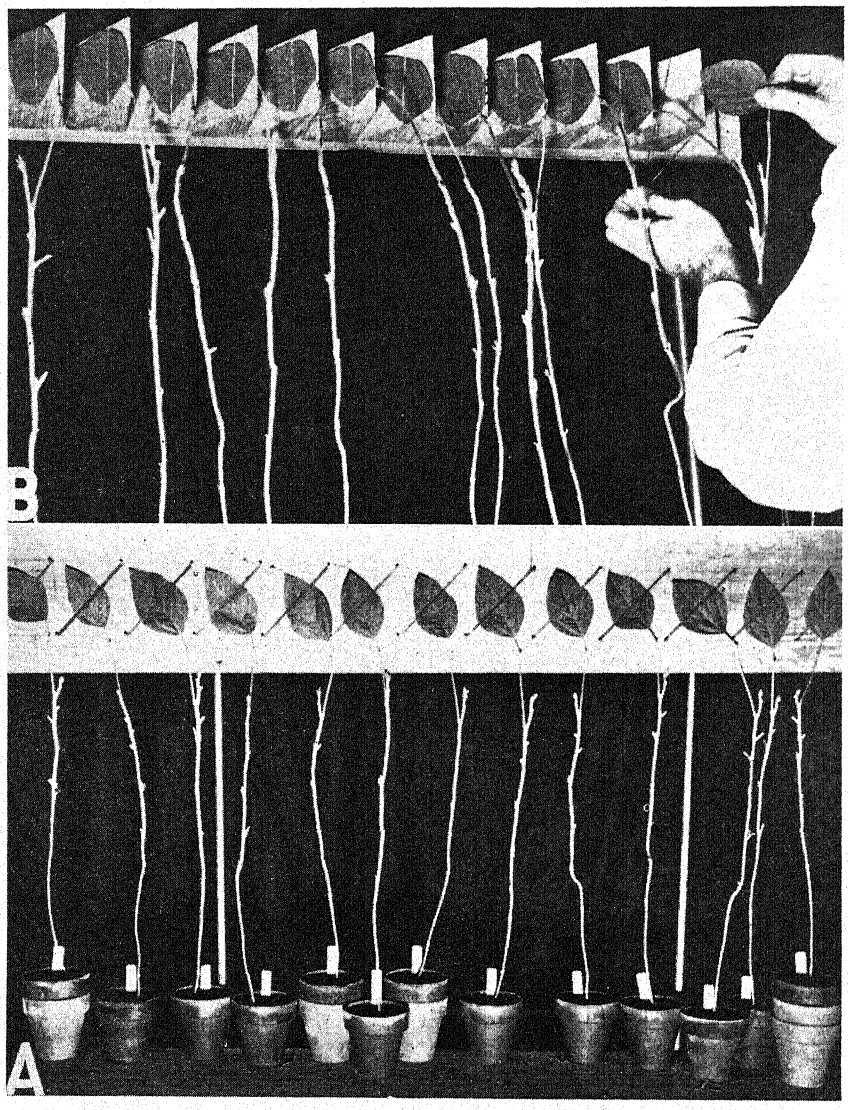


FIG. 3.—Methods employed to hold leaflets in image plane. *A*, setup of soybean leaflets held flat in image plane. *B*, setup of soybean leaflets held on wedges at angle of 30° to light beam. This type of setup used in orange-red region of spectrum and flat type was used in blue-violet region.

Experimentation and results

Reciprocity relationships, overnight threshold intensities, effect of leaf positions, and irradiation at various times after the start of the dark period

In experiments with the spectrograph different energies of irradiation for a particular wave-length region were given by varying time of exposure at constant intensity rather than by varying the intensity. With either time or intensity varying, it was necessary to test the reciprocity law, which states that the product of the duration and the intensity of light that will produce a certain effect is a constant. The validity of the spectrograph procedure required that the inhibiting effects on flowering caused by equal energies produced by such variations be constant within reasonable limits; that is, the procedure required that the reciprocity law hold for the factor of time and intensity over a range of approximately 1–60 minutes.

The reciprocity law was tested with both soybeans and cockleburs. The plants in all cases were defoliated in the manner previously described for the spectrograph experiments. They also received the same photoperiodic treatment; that is, the soybeans received six cycles of 10 hours of light and 14 hours of dark, and the cockleburs, three cycles of 12 hours of light and 12 hours of dark. The dark-period interruptions were near the middle in each case.

In any given experiment a single light source was used for the dark-period interruptions, and the desired intensities at the leaf surface were obtained by placing the plants at appropriate distances from it. The light source could have been any type that gave visible radiation in an effective region. In one experiment with Biloxi soybeans (table 1), an incandescent-filament lamp was used with a no. 244 Corning H.R. Lantern-red filter of standard $\frac{1}{4}$-inch thickness. Three groups of seven lots of ten plants each were included in the experiment. These groups received dark-period interruptions of 2, 6, and 18 minutes, respectively. The seven lots of each group were subjected to intensities such that corresponding lots of each group received the same total energies and successive lots within groups received progressively greater energies. The experiment included one lot of ten control plants which received no dark-period interruptions. With increasing energy in each group there was generally a decrease in number of flower primordia, the greatest changes occurring between the first and second and

TABLE 1

Biloxi soybean reciprocity experiment in which intensities were adjusted so that several different quantities of energy were each applied in three different durations of treatment. Red light from a no. 244 Corning glass filter in front of a 300-watt incandescent-filament lamp used for dark-period interruptions

Lot no.	Relative energy	No. of flower primordia formed on ten plants following different durations of dark-period interruption*		
		2 min.	6 min.	18 min.
1	1.00	24	23	14
2	1.67	16	8	14
3	2.34	6	4	6
4	3.00	7	0	4
5	3.64	2	1	0
6	4.31	0	0	0
7	5.00	0	0	0

* Controls had thirty-two flower primordia on ten plants.

the second and third levels, but floral initiation in corresponding lots of the three groups was about the same at corresponding energy levels. This indicates that, within the limits of time and intensity tested in this experiment, the reciprocity law held as closely as could be expected from the type of measurements performed with such biological material.

Reciprocity experiments on Biloxi soybeans similar to the above were also carried out with filtered radiation from a 600-watt G.E., a.c. Uviarc. The radiation was chiefly restricted to the mercury lines at 5461, 4358, and 4047 A. Energies required to suppress initiation of flower primordia were equivalent within a factor of two for exposures of 5, 12, 15, and 30 minutes. These times encompass the ones later used in determining the action curve for the violet and blue portions of the spectrum.

A reciprocity experiment with cocklebur was conducted in which the source of radiation for the dark-period interruptions was an unfiltered incandescent-filament lamp. The intensities of radiation were so adjusted that duration of interruption could be varied from 30 seconds to 64 minutes at seven constant-energy levels that differed from one another by a factor of slightly less than 1.5. The six plants of each experimental lot were subjected to dark-period interruptions within a period starting an hour before and ending an hour after the middle of the dark period; that is, during the sixth and seventh hours after the start of the 12-hour dark period.

Floral initiation was prevented in half or more of the plants of all but three lots at relative energy levels of 4.0 or higher (table 2). In all lots that received lower relative energies, on the contrary, two-thirds or more of the plants initiated flowers. Thus, the energies required to suppress floral initiation in the various groups were constant within the factor of 1.5 used between successive lots of a particular group.

Determinations were made for soybeans of the minimum intensity of illumination necessary to suppress floral initiation when given throughout the entire 14-hour dark period. In these experiments lots of plants were placed at various distances from a low-wattage incandescent-filament lamp in the same way as for one irradiation time of a reciprocity experiment. Three experiments, in each of which several lots of ten plants were used, gave the threshold intensity as 0.01, 0.02, and 0.02 foot-candles, respectively. The corresponding energies received by the leaflets of the plants were the same, within a factor of 3, as those required to prevent floral initiation when they were given in short photoperiods near the middle of the dark period.

The overnight threshold intensities for suppression of initiation of flower primordia by soybeans and cockleburs were directly compared. The soybeans were illuminated with unfiltered incandescent radiation for each of 6 nights of 14 hours as usual, and the cockleburs in two lots for 3 and for 6 nights of 12 hours each. The threshold intensity for the soybeans in this experiment was 0.01 foot-candles; that for the first lot of cockleburs was 0.01 foot-candles and for the second 0.02 foot-candles. Thus the two species, when compared under these conditions, required about the same intensity of continuous illumination to prevent floral initiation.

Overnight experiments were also carried out with violet light obtained by filtering the radiation from a 300-watt incandescent lamp through a no. 511 Corning Lantern-blue filter of $\frac{1}{4}$-inch thickness. Fifteen soybean plants placed 2 meters

from the light produced thirty-one flower primordia while fifteen plants held in the dark for the 14-hour nights produced forty. These results indicated that such a light had little if any effect in inhibiting floral initiation and that it could therefore be used as a safe-light during the setting-up of plants for the various experiments. To provide a still greater factor of safety, a 100-watt incandescent lamp was substituted for the 300-watt one. This lamp gave illumination sufficient for necessary manipulations and identifications of plants.

Leaflets of soybean plants in the reciprocity and threshold experiments were placed so that the surfaces were oriented between normal and 45° to the light beam. In the experiments with the spectrograph, leaflet surfaces were placed normal or at an angle of 30° to the light beam. Two experiments were conducted to test the relative effectiveness of irradiation under these various conditions. When the leaflets were placed at 30°, about twice as much energy was required as when the leaflets were placed normal to the light beam.

Since in the spectrograph experiments several lots were treated during a 2-hour period, it was necessary to know whether or not the energy required to prevent floral initiation was constant throughout this period. To obtain data on this point, a succession of threshold experiments was performed in which each experiment was started at a different time during the dark period. The intensity ranges were so adjusted that durations of interruption were 2–4½ minutes for soybean and 4–9 minutes for cocklebur. The energy required to prevent floral initiation in Biloxi soybean (table 3) was constant over the period from 1 hour before until 2 hours after the middle of the dark period. Results of two other similar tests indicated that about one and a half times more energy was required to suppress formation of flower primordia if applied at the end of 6 hours after the beginning of the dark period than if applied at the end of 7 or 8 hours. In view of these results all the dark-period interruptions for the spectrograph on soybeans were performed within a 2-hour interval starting

TABLE 2

COCKLEBUR RECIPROCITY EXPERIMENT IN WHICH INTENSITIES WERE ADJUSTED SO THAT SEVERAL DIFFERENT QUANTITIES OF RADIANT ENERGY WERE EACH APPLIED IN EIGHT DIFFERENT DURATIONS OF TREATMENT. WHITE LIGHT FROM INCANDESCENT-FILAMENT BULB USED AS LIGHT SOURCE

Lot no.	Relative energy	No. of plants per lot of six that formed flower primordia following different durations of dark-period interruption*								
		30 sec.	1 min.	2 min.	4 min.	8 min.†	8 min.†	16 min.	32 min.	64 min.
1	1.00	5	6	6	6	6	6	6	6	6
2	1.41	6	6	6	5	6	4	6	6	6
3	2.00	6	6	6	6	4	4	6	5	6
4	2.83	6	6	6	4	4	6	5	4	4
5	4.00	4	0	0	4	4	0	1	1	2
6	5.66	2	3	0	0	0	0	1	1	0
7	8.00	.....	2	0	1	2	0	0	0	0

* All uninterrupted controls flowered.

† Light source for first five exposure times differed from that of the last four. An 8-minute lot was run with both sources.

30 minutes before the middle of the 14-hour dark period.

Similar experiments conducted for cocklebur (table 4) gave essentially the same results. Results obtained during an interval of $2\frac{1}{4}$ hours beginning at the middle of the dark period were practically constant. This was verified in four other experiments. The spectrograph experiments were restricted to the 2-hour period immediately following the middle of the 12-hour dark period.

A striking feature of these experiments was the asymmetry in plant response with respect to the middle of the dark period. Results presented in tables 3 and

TABLE 3

EFFECT ON BILOXI SOYBEAN OF IRRADIATION WITH WHITE LIGHT AT SEVERAL ENERGY LEVELS AND AT VARIOUS TIMES AFTER START OF 14-HOUR DARK PERIOD
DURATIONS OF IRRADIATION RANGED FROM 4 TO 9 MINUTES

RELATIVE ENERGY	NO. OF PRIMORDIA FORMED PER LOT OF EIGHT PLANTS FOLLOWING IRRADIATION AFTER VARIOUS NUMBERS OF HOURS FROM START OF DARK PERIOD										
	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0	10.5	11.0	11.5
1.00	.....	.....	.....	.....	9	13	13	17	.....	.....	.....
1.50	.....	.....	.....	6	9	10	9	13	15	.....	.....
2.25	.....	.....	.....	4	10	9	7	15	15	.....	.....
3.40	.....	.....	.....	2	2	1	1	8	10	13	.....
5.10	4	5	6	0	1	0	0	4	2	9	10
7.60	8	5	2	0	0	0	0	1	1	7	9
11.40	7	7	1	0	0	0	0	0	0	1	11
17.00	2	0	0	0	.....	.....	.....	.....	0	0	4
25.60	1	0	0	.....	.....	.....	.....	.....	.....	0	9
38.40	0	0	0	.....	.....	.....	.....	.....	.....	0	2
57.60	0	0	0	.....	.....	.....	.....	.....	.....	.....	4
Controls*	7				16	12		22			

* Experiment conducted with plants in four different control rooms. Each group to be compared with its own control.

TABLE 4

EFFECT ON COCKLEBUR OF IRRADIATION WITH WHITE LIGHT AT SEVERAL ENERGY LEVELS AND AT VARIOUS TIMES AFTER START OF 12-HOUR DARK PERIOD
DURATIONS OF IRRADIATION RANGED FROM 2 TO $4\frac{1}{2}$ MINUTES

RELATIVE ENERGY	NO. OF PLANTS PER LOT OF EIGHT FORMING FLOWER PRIMORDIA FOLLOWING IRRADIATION AFTER VARIOUS NUMBERS OF HOURS FROM START OF DARK PERIOD*							
	3.75	4.50	5.25	6.00	6.75	7.50	8.25	9.00
1.00	.....	8	8	8	8	8	.....	.....
1.50	8	8	8	8	8	8	8	.....
2.25	8	8	8	8	8	8	6	8
3.40	8	8	8	4	2	2	5	5
5.10	8	7	4	2	1	1	3	5
7.60	7	4	2	1	1	0	1	4
11.40	4	3	0	1	0	0	2	3
17.00	3	.....	.....	.....	.....	.....	1	1
25.60	.....	.....	.....	.....	.....	.....	.....	1

* All controls on short day flowered.

4 are representative of others obtained. They show, for instance, that at least three times as much energy was required to suppress floral initiation of cocklebur when given 1.5 hours before the middle of the dark period as contrasted with 1.5 hours after the middle. Soybeans exhibited a similar difference in sensitivity to treatments at 3 hours on either side of the middle of the dark period. In general, the asymmetry for both plants was such that markedly greater energy was required to suppress floral initiation when the radiation was given appreciably before rather than after the middle of the dark period.

If the time of interruption was so placed as to permit a portion of the dark period to approximate that required for flowering, the response was variable. This for soybean (table 3) was illustrated by the strikingly different behavior following interruption at 11 or at 11½ hours after the start of the dark period. Similar results were obtained in experiments undertaken to determine the critical day-length necessary for flowering of cocklebur.

Limits of effectiveness in the spectrum

While spectrograph experiments were performed with high energies at wave lengths as great as 9000 A., it was still considered necessary to test further for possible effectiveness of infrared radiation in preventing floral initiation. This was done by irradiating fifteen soybean plants throughout the night at 1.2 meters from a 300-watt lamp behind a Corning no. 254 filter of ¼-inch thickness which did not transmit wave lengths shorter than 7500 A. These were compared with fifteen other plants that received no dark-period interruptions. All plants of each lot formed flower primordia, and there was no difference between lots in the number of primordia formed. Only about 10 seconds' irradiation in the region of 6500 A. under these conditions would have been required to prevent initiation of primordia.

Spectrograph experiments were not completely satisfactory in establishing the nature of the photoperiodic response to radiation in the near ultraviolet. This was caused by a probable lower or possible zero effectiveness of such radiation, by the steadily decreasing intensity in the radiation source at shorter wave lengths, by the increased dispersion of the spectrograph, and by the decrease in transmission of the prisms. For these reasons tests were made with the 3650 A. mercury line isolated by means of filters. The radiation source was a 600-watt G.E., a.c. Uviarc equipped with no. 586 and no. 738 Corning filters, each of ¼-inch thickness. Ten soybean plants exposed for 1 hour at 1.0 meter from this source initiated twenty flower primordia, while ten control plants that received no dark-period interruption initiated twenty-four primordia. Thus at 3650 A., energies as great as 2000 kiloergs per cm.^{2} did not inhibit the formation of flower primordia, whereas lower energies were inhibitory in even the least effective region of the visible spectrum.

Action spectrum for prevention of floral initiation by Biloxi soybeans

After a number of preliminary tests, fourteen spectrograph experiments were performed to determine energies required at various wave lengths to suppress initiation of flower primordia by Biloxi soybeans. Six of these were designed primarily for the violet-blue portion of the spectrum, but they included a considerably wider region. The maximum

time of exposure was 21 minutes, and each exposure was repeated on four lots of plants.

In one experiment in this region (fig. 4), 220 plants were irradiated at fourteen different wave-length regions and for six different durations. The resulting energies at each wave-length station are plotted on the graph. Superimposed on these energy lines are the total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. A response curve was drawn freehand through the energy lines, crossing them between points at which sharp breaks occurred in the numbers of flower primordia produced. The maximum energy required to prevent floral initiation in this region was about 1200 kiloergs at 4800 A. Below this wave length the response curve approximately followed the radiation curve (i.e., 21-minute exposures were more effective at all wave lengths than 14-minute exposures). Thirty-six plants were maintained as short-day controls. These developed an average of 2.4 flower primordia each, or 9.6 for a group of four plants. The response curve of a second experiment

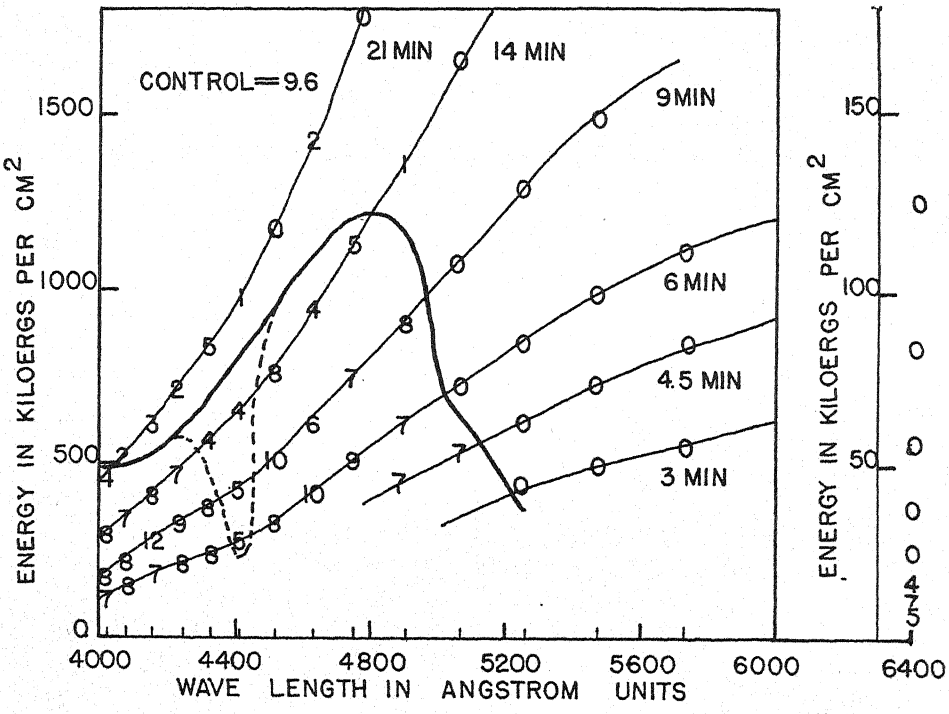


FIG. 4.—Response curve for suppression of floral initiation in Biloxi soybean in blue-violet region of spectrum. Leaflets were held at fourteen different wave-length stations for six different durations. Resulting energy at each wave-length station is plotted, and superimposed on energy lines are total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. Response curve was drawn freehand through energy lines, crossing them between points at which sharp breaks occurred in number of flower primordia produced. Dotted portion of curve at 4400 A. suggests the possibility of an enhanced effect in this region. Results of an experiment to compare effectiveness of radiation at 6400 A. with that in blue-violet region are shown at extreme right of figure.

(fig. 5), extending to slightly shorter wave lengths, had the same general features of that of figure 4. In both these experiments and in one previously described (15), radiation near 4400 A. was perhaps somewhat more effective in preventing floral initiation than was that of neighboring regions. No evident effect of the type suggested by the dotted line of figure 4 was observed in the other experiments in the violet-blue portion of the spectrum. If such an effect occurs in this region, it is close to the limit of detection imposed by the method of measurement and the variability of the biological material.

Response curves taken from the six experiments in which the violet-blue portion of the spectrum was used (fig. 6) show approximately a twofold variation between the extreme curves. The relative response in the various wave-length regions for a particular experiment was, of course, far less variable; that is, the shape of a response curve was determined very accurately within each experiment, but the absolute value of the ordinates varied somewhat from one experiment to another. This variability between experiments was the result of the variable environmental conditions under which the plants were produced in the green-

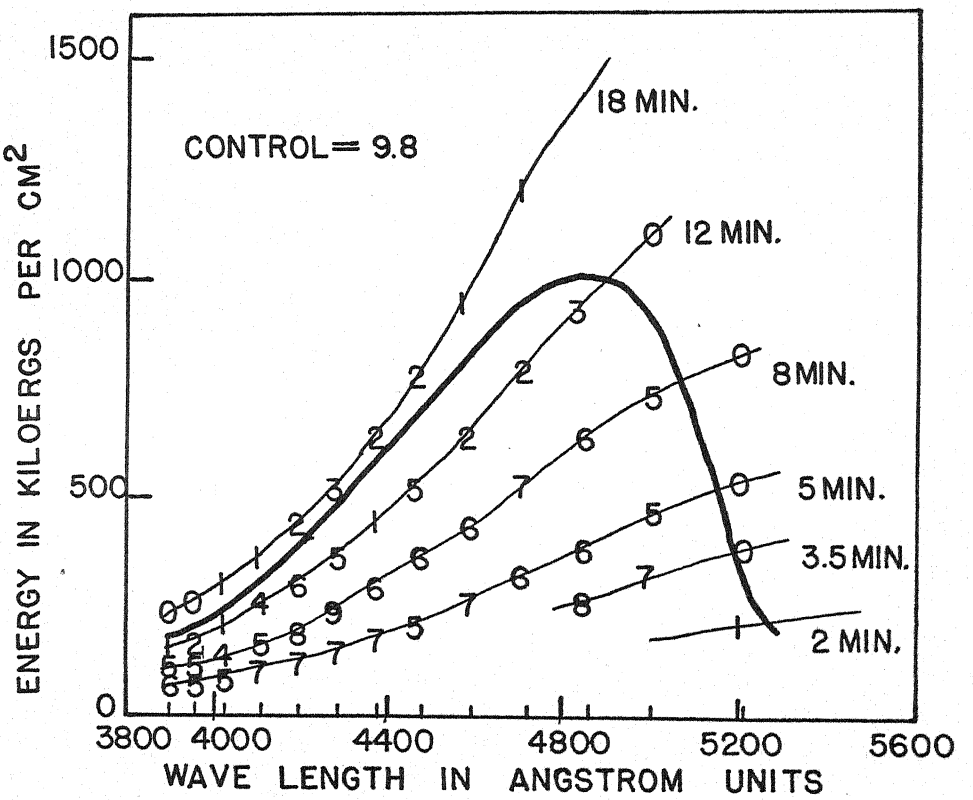


FIG. 5.—Another response curve for suppression of floral initiation in Biloxi soybean in blue-violet region of spectrum.

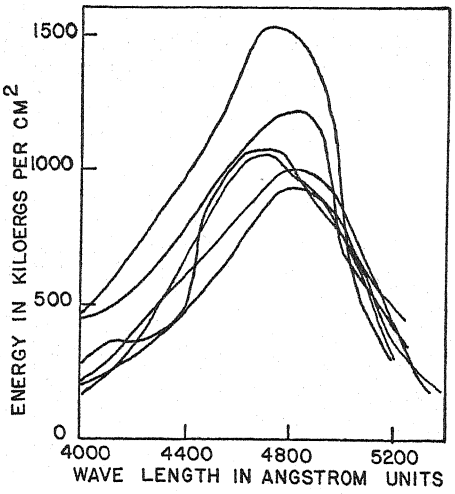


FIG. 6.—Response curves for suppression of floral initiation in six different experiments with Biloxi soybean in blue-violet region. There is approximately a twofold variation between extreme curves.

house, since experiments were performed at all seasons of the year.

Seven experiments were performed in the region between 5400 A. and 7800 A. For these experiments the leaves were held against the wedges at an angle of 30° to the light beam, as previously described.

In one experiment (fig. 7) the range from 5500 A. to 6800 A. was covered by stations the centers of which were 5.5 cm. apart. The range from 6800 A. to 7900 A. was covered by five stations that were 3.75 cm. apart. A narrow slit was used in the spectrograph in order that the effective slit width could be maintained at about one-half the width of the leaf projected along the light beam. The solid curve (fig. 7) was taken as the one representing the plant response for com-

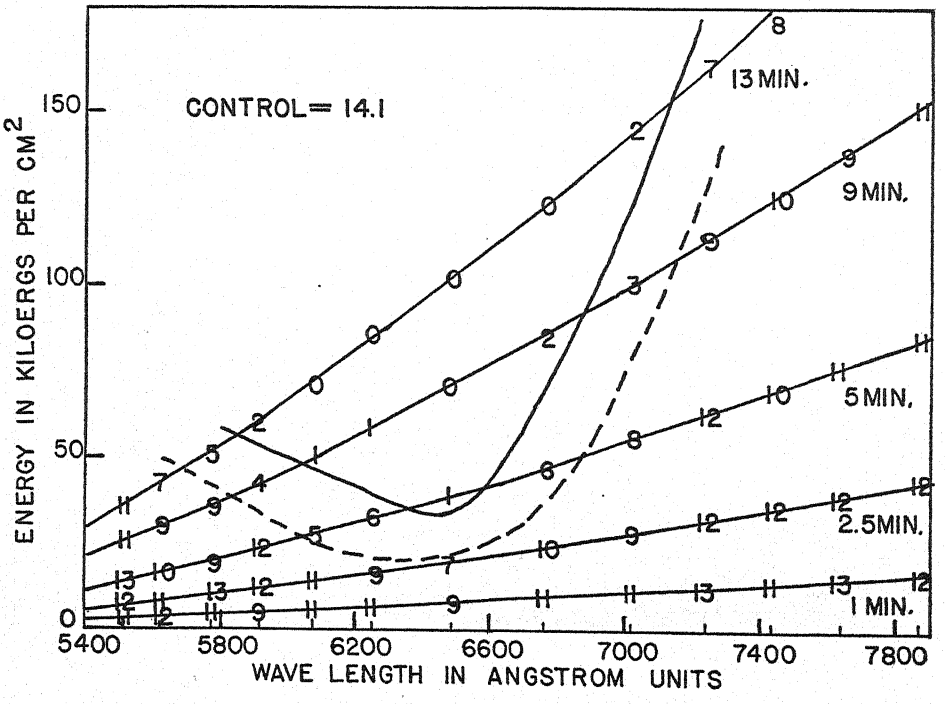


FIG. 7.—Response curve for suppression of floral initiation in Biloxi soybean in range from 5500 A. to 7900 A. Solid curve was taken as one representing the response, while broken-line curve illustrates gradation effect with decreasing energy.

parison with the action curve in the violet-blue portion of the spectrum. It followed the energies required at the various stations for practically complete suppression of flower primordia. A dotted curve below this one illustrates a gradation effect of decreasing energy.

The long-wave-length limit of radiation effective in preventing floral initiation was established by four spectrograph experiments. One of these (fig. 8) was particularly designed to trace the response curve as far toward long wave lengths as was feasible. Approximately 2300 kiloergs per sq. cm. were required to suppress primordium development at 7300 A. However, some effect on floral initiation was possibly caused by high energies at 7700 A.

Response curves for seven separate experiments (fig. 9) in the yellow-red portion of the spectrum show very close agreement in the region for maximum

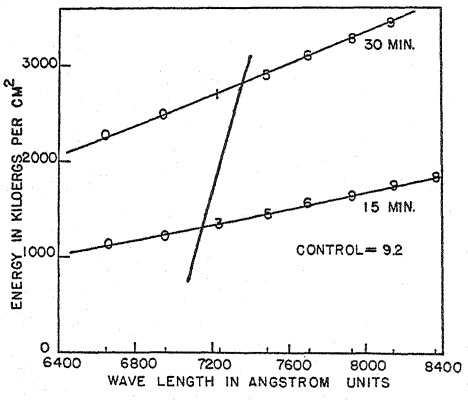


FIG. 8.—Results of one of the experiments to establish long-wave-length limit of radiation effective in preventing floral initiation.

effectiveness. One of these curves covered wave lengths down to about 5400 A. and served to tie this group of experiments to those of the violet-blue region of the spectrum which covered the range of approximately 4000 A. to 5400 A. The region covered by this particular curve was one over which the effectiveness of radiation was changing rapidly.

Energies required to suppress development of flower primordia of Biloxi soybean at the most effective regions in the

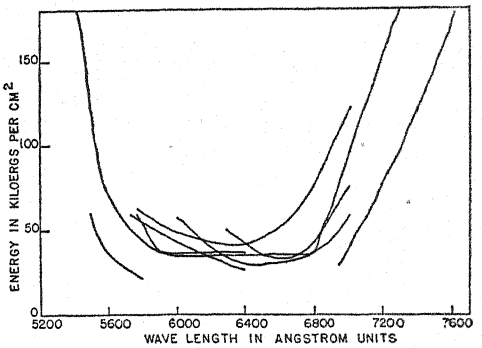


FIG. 9.—Response curves for seven separate experiments in yellow-red portion of spectrum.

red and the violet and at the least effective region in the blue portion of the spectrum are shown in table 5. The most direct comparison between these values was given by the experiments of 9–26–45 and 9–17–45, which were designed to compare effectiveness of radiation in a narrow region of the red with that in the blue-violet region of the spectrum. These experiments made use of the previously described lens arrangement at the red end of the spectrum centered at 6400 A., which permitted all leaves to be placed normal to the light beam and increased the radiation times in the red to the same order as those of the violet-blue. The data of the experiment of 9–26–45 (table 5) are shown with the results obtained at 6400 A. on an ordinate one-tenth that used for the rest of the data (fig. 4). In this experiment the energy required to prevent floral initiation at 4800 A., the least effective region in the visible, was about sixty times as great as the energy required at 6400 A.

TABLE 5

SUMMARY OF ENERGIES REQUIRED TO PREVENT FLORAL INITIATION IN BILOXI SOYBEAN IN MOST EFFECTIVE REGIONS OF RED AND VIOLET AND IN LEAST EFFECTIVE REGION OF BLUE

DATE	KILOERGS PER SQ. CM.			LEAF POSITION IN RELATION TO LIGHT BEAM
	Red	Blue	Violet	
9–26–45.....	20	1200	450	Normal
9–17–45.....	25	1500	500	Normal
7–10–44.....	40	.....	.....	30°
6– 5–44	27	.....	.....	30°
5–29–44.....	34	.....	.....	30°
5–22–44.....	36	.....	.....	30°
5– 8–44.....	24	.....	.....	30°
9– 4–44.....	.....	900	200	Normal
8–22–44.....	.....	1000	220	Normal
7–31–44.....	.....	1100	300	Normal
2–27–45.....	.....	1000	200	Normal

The data of all experiments with Biloxi soybeans were combined to give an action spectrum (fig. 10). The experiments of 9–26–45 and 9–17–45 (table 5) were very helpful in establishing a common ordinate for the two halves of the curve, a matter concerning which there had previously been a twofold factor of uncertainty (15).

ACTION SPECTRUM FOR PREVENTION OF FLORAL INITIATION BY XANTHIUM SACCHARATUM

The action spectrum for *Xanthium* was determined from the results of four experiments in the red portion of the spectrum and four in the blue. The detailed data presented for certain of those

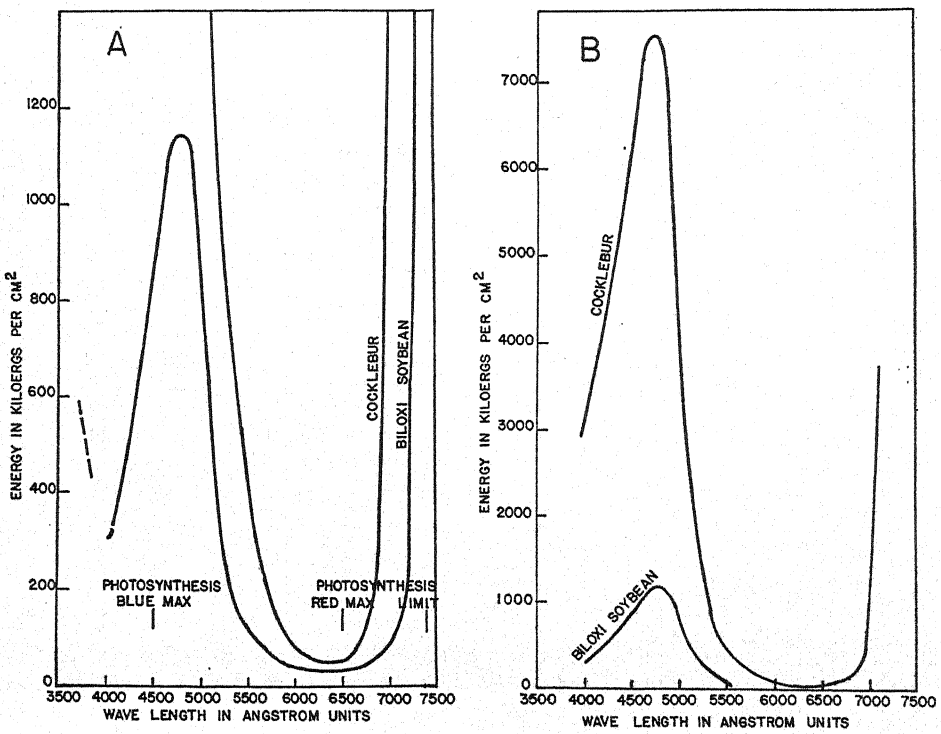


FIG. 10.—Composite action spectrum for suppression of floral initiation in soybean and cocklebur. *A*, points on soybean curve give energy required at middle of a 14-hour dark period to prevent floral initiation. Wave lengths for maximum photosynthesis and wave-length limit as measured by HOOVER (8) are shown. *B*, points on cocklebur curve give energy required at middle of a 12-hour dark period to prevent floral initiation.

experiments (figs. 11, 12, and 13) were expressed in terms of the number of plants per lot of four that initiated flower primordia following treatment.

The action curves (fig. 14) for the four experiments in the red region, based upon about 1200 plants, agreed with one another within reasonably narrow limits between 5400 A. and 6900 A. The broken-line curve approximates a mean for the four experiments, although it gives relatively little weight to the one response curve that shows lowest energy between 5500 and 6600 A.

Since the experiments with cocklebur in the red region were performed with the carbon-arc lamp, the times of exposure to produce a given amount of energy were very much shorter than those for

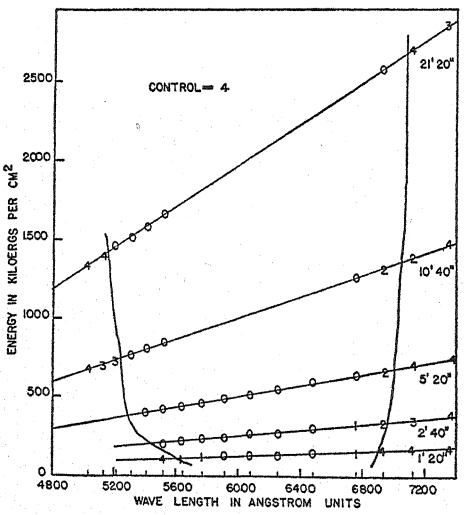


FIG. 11.—Response curve for prevention of floral initiation in cocklebur. Figures superimposed on energy lines indicate number of plants from each lot of four that formed flower primordia.

the soybean experiments performed with the projection lamp (fig. 7). One of these cocklebur experiments (figs. 11 and 12), which was performed with energies as great as 2500 kiloergs per sq. cm., showed that radiation at wave lengths longer than 7000 A. was very ineffective in preventing floral initiation in cocklebur. This limit thus occurred at a somewhat shorter wave length for cocklebur than

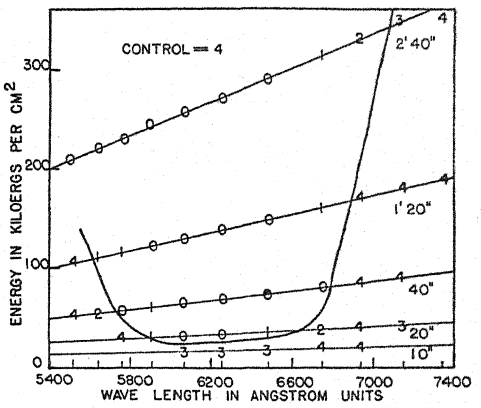


FIG. 12.—Continuation of lower part of curve shown in fig. 11. Region of maximum effectiveness for preventing floral initiation in cocklebur was about 6300 A.

for soybean. The region of maximum effectiveness for cocklebur was about 6300 A. and was somewhat more sharply defined than in the case of soybean.

Preliminary experiments indicated that it would be difficult to obtain adequate energy in the blue portion of the spectrum to permit tests equivalent to the ones used for soybeans. The energy in the specific wave-length regions was accordingly made as great as the limiting factors permitted by reducing the distance to the image plane, broadening the slit, running the arc at the maximum power input, and increasing the time of irradiation as much as was feasible. The resulting decrease in dispersion and resolving power was not sufficiently great to interfere with the results. Under the conditions employed floral initiation of cocklebur could be suppressed with exposures of 50 minutes in the blue-violet region from 3900 A. to 4800 A. and with

considerably shorter time at longer wave lengths. With such long exposures it was not possible to treat more than four plants per station during the 4-hour duration of an experiment. However, since

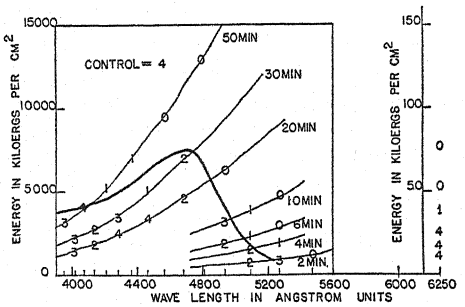


FIG. 13.—Response curve for prevention of floral initiation in cocklebur in blue-violet portion of spectrum. Data on extreme right show results of an experiment that was used to compare effectiveness in blue-violet region of spectrum with that in red region.

the dispersion was so great in the blue-violet region from 3900 A. to 4800 A., adequate sampling was attainable by confining the 50-minute treatments to five alternate stations. The intervening stations in this range and all stations above 4800 A. were used for the shorter exposures (fig. 13). The region of least effectiveness was near 4800 A., and this region was also found least effective in soybean, but the energies required to prevent floral initiation were far greater for cocklebur.

Response curves from the four experiments performed in the blue-violet region were very similar (fig. 15) and resulted from experiments performed with different lots of plants grown during the period of May–August, 1945. Any variability between these lots did not alter the general character of the curves for the action spectrum.

Direct comparison was made of the effectiveness of radiation in the blue-violet portion of the spectrum with that of the most effective part of the red in the same way as described for soybeans. These comparisons were made during the course of the four experiments in the blue region. The energy ratios between the least effective region of the blue and the most effective portion of the red (table 6) were 250:1, 150:1, 200:1, and 220:1 in the four experiments. These ratios were above three times greater than found for soybeans, owing chiefly to lower effectiveness of the blue radiation. Aside from this, the responses of soybeans and cockleburs were closely similar, as shown in the composite curve of the action spectrum (fig. 10*B*).

Discussion

Experiments reported were undertaken to gain information about the initial step in the prevention of flowering

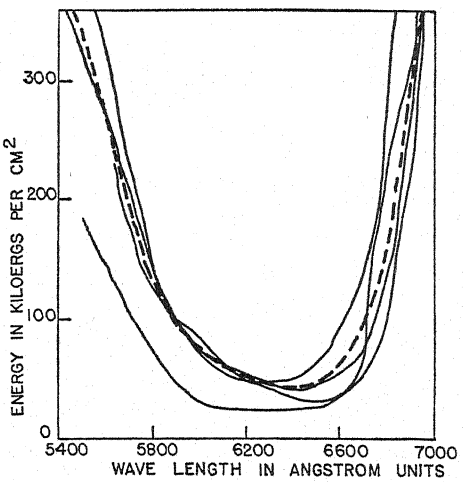


FIG. 14.—Response curves for cocklebur from four experiments in yellow-red region of spectrum are shown by solid curves. Broken-line curve shows average selected from this group.

in short-day plants by the action of light. Many workers (4, 12, and 13) have shown that the photoperiodic reaction occurs in the leaves and that it can be controlled by the action of visible

radiation. It follows, therefore, that some colored compound in the leaf is involved in the initial step of this photoreaction. This compound might be closely allied to the material that is transported to the meristem where it regulates initiation, or it might be one of the common leaf pigments which enters into photochemical reactions thereby preventing the appearance of a regulatory substance at the terminal meristem.

The simplest observation to be made from the data is that both Biloxi soybean and cocklebur have action curves that are similar in many respects. Both plants respond photoperiodically to radiation from all portions of the visible spectrum, but the energy required to prevent floral initiation in either plant varies greatly over the spectrum. Positions of minimum and maximum effectiveness in the spectrum and the long-wave-length limit of response are the same for both plants. This, at least, is an

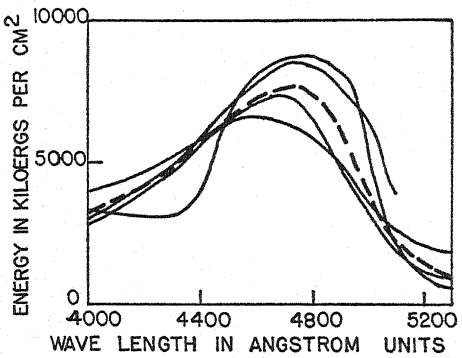


FIG. 15.—Individual action spectra for four experiments with cocklebur in blue-violet portion of spectrum are shown by solid curves. Selected average is shown by broken-line curve.

indication that the same pigment or pigments are involved in the photoperiodic response of the two plants.

The ideal case for studying a biological response to light is one in which the action curves can be corrected for scattered light and for absorption by screening pigments, the latter requiring a priori knowledge about the effective pigment. If possible, the system should not absorb too much of the incident energy. These conditions are not realized in measurement of the photosynthetic action curve for higher plants because of leaf structure but have been attained for unicellular green algae (6). In the case of photoperiodic control of flowering no method has yet been devised to avoid the complexities of light absorption in the leaves of the plants.

TABLE 6

SUMMARY OF ENERGIES REQUIRED TO PREVENT FLORAL INITIATION IN COCKLEBUR IN MOST EFFECTIVE REGIONS OF RED AND VIOLET AND IN LEAST EFFECTIVE REGION OF BLUE

DATE	ENERGY IN KILOERGS PER SQ. CM.			LEAF POSITION IN RELATION TO LIGHT BEAM
	Red	Blue	Violet	
12- 4-44....	60	.....	.....	Normal
6- 6-45....	50	.....	.....	30°
7- 3-45....	25	.....	.....	30°
7-18-45....	30	.....	.....	30°
8-21-45....	30	7500	3500	Normal
7- 7-45....	55	8500	2500	Normal
5-31-45....	50	10000	3000	Normal
8- 7-45....	30	6500	2500	Normal

The possibility that the action curve might be an expression of absorption by a common leaf pigment will be examined in detail. Pigments in question are carotinoids and chlorophylls *a* and *b*. The action spectra indicate effectiveness of light at wave lengths as long as 7000 Å. in controlling floral initiation and thus definitely rule out the first class of compounds other than in their screening action. The features of the action curve that suggest that chlorophyll may be the effective pigment are the long-wave-

length limit, the general effectiveness throughout the visible, the most rapid change in response at the steepest portion of the chlorophyll-absorption curve, and the presence of two maxima of response, one in the red and the other in the violet. Many factors must be discussed in considering the possibility that chlorophyll may be the effective pigment, and, in the end, decision must remain for future work.

Familiar action curves involving chlorophyll as the effective pigment are those for photosynthesis. These curves have been determined for wheat by HOOVER (8) and for *Chlorella* suspensions by EMERSON and LEWIS (6). In the former case the amount of CO_2 fixation for unit incident energy varies but little across most of the visible spectrum, which is to be expected with great amounts of the absorbing pigment in the light path. In the latter investigation the oxygen evolution was shown to follow the absorption spectrum of chlorophyll with constant quantum efficiency. Expressed in another way, maximum photosynthesis for dilute *Chlorella* suspensions was obtained for unit incident energy at the absorption maxima and less in the more transparent regions (EMERSON and LEWIS, fig. 7). Both curves, however, have the same long-wave-length limit of response. This limit, moreover, is the long-wave-length limit of light absorption by isolated chloroplasts (5). It corresponds within the limit of experimental error to the long-wave-length limit for photoperiodic response of soybean and cocklebur, and this correspondence might be expected irrespective of the detailed nature of the two processes provided the red absorption bands are effective in both.

Photosynthetic effectiveness for wheat reaches a slight maximum near 4400 A. and decreases greatly between 4400 and 4000 A. The maximum effectiveness for stopping floral initiation apparently takes place at shorter wave lengths. About half the experiments with the violet-blue portion of the spectrum suggest an enhanced effect of radiation near 4400 A., as indicated by the dotted line on figure 4. The least effective portion of the spectrum is near 4800 A. for both soybean and cocklebur. The two species, however, differ significantly in the effectiveness of such radiation in comparison with the most sensitive region in the red. Ratios of energies required to stop floral initiation are:

Soybean E (4800 A.):E (6400 A.) = 60:1
Cocklebur E (4800 A.):E (6400 A.) = 200:1

This region of minimum photoperiodic effectiveness is near the wave lengths at which chlorophylls *a* and *b* have their maximum absorption coefficients.

If chlorophyll is the pigment responsible for the initial step of photoperiodic control of flowering, then the immediate reaction takes place in the chloroplasts, since absorbed energy can be transferred only a distance of a few molecular diameters. The absorbed energy effective in preventing flowering of short-day plants is probably transferred to some compound appearing in a chain of chemical reactions that do not depend upon absorbed light. In the absence of light a product of these reactions reaches sufficient concentration so that the amount diffusing away from the chloroplasts and finally reaching developing meristematic tissue is sufficient to control development. The compound to which the energy is transferred might thus undergo a side reaction, such as oxidation, which would break the chain of reactions lead-

ing to the final effective compound. The break could be at any point along the chain, including the final reaction.

If chlorophyll is the effective pigment, then possibly the chlorophyll-containing cells must absorb enough energy to reduce the concentration of a compound entering the photoreaction to some definite but unknown average level. Radiation in the region of maximum absorption will be more reduced in intensity at the chloroplasts farthest from the irradiated surface than will radiation having less absorption. If the energy received is below a certain value, these particular chloroplasts will still supply the active compound. In regions of low absorption of light by the chloroplasts, near 5600 A., for example, radiation is less reduced at the chloroplasts farthest from the irradiated surface, and the actual energy absorbed is possibly greater than for the region of the maximum absorption coefficient. As a result less energy could be required, as actually observed, for suppression of floral initiation at 5600 A. than at 4800 A., where the absorption coefficient is greater.

Change of light absorption with depth in an absorbing medium might be considered in greater detail to illustrate the effect. The intensity at a particular depth (x) is given by

$$I = I_0 e^{-ax},$$

where I_0 is the incident intensity and a is the absorption coefficient. Energy absorbed at a particular depth is given by the derivative of the above expression with respect to depth, or

$$\frac{dI}{dx} = -aI_0 e^{-ax}.$$

At the surface of the absorbing medium x is zero and dI/dx is $-aI_0$. Thus the energy absorbed at the surface for a given incident energy depends directly upon the absorption coefficient. As the depth increases ($x>0$), the absorption decreases, that is, e^{-ax} decreases with increasing values of x, but at a different rate for each absorption coefficient (a). The rate of change of absorption decreases as the absorption coefficient decreases, and a depth is finally reached at which absorption of strongly absorbed radiation becomes less than that of weakly absorbed radiation. This change of absorption with depth in a homogeneous medium is illustrated in figure 16 for three absorption coefficients corresponding to three different wave lengths of incident light having the same intensity I_0 at each wave length. At a depth of 2.5 units ($x=2.5$) about ten times as much energy is absorbed per unit of volume for an absorption coefficient of 0.5 as for 2.0. At the surface, of course, the absorption was four times as great for the latter as for the former. In general, the effect of self-screening by the chloroplasts or within a chloroplast could be of the type to broaden the region of relative great sensitivity in the red, orange, and yellow, as contrasted with the absorption spectrum of chlorophyll, and to reduce the effectiveness of the blue.

Consideration of phototropic movement of plants is of interest in contrast to photoperiodic control of flowering. The action curve for the bending of the Avena coleoptile was first measured by BLAAUW (1) and later by JOHNSTON (9). Both observers found a maximum bending response in the neighborhood of 4400 A. with a second near 4750 A. Radiation beyond 5500 A. does not produce bending. WALD and DU BUY (17) indicated that the effective pigment is probably a carotinoid, and, by extracting oat coleoptiles, they obtained a solution having

an absorption spectrum similar to the action curve. The initial step is the absorption of light by the carotinoids of the plant, but control of bending is brought about through the action of auxins which

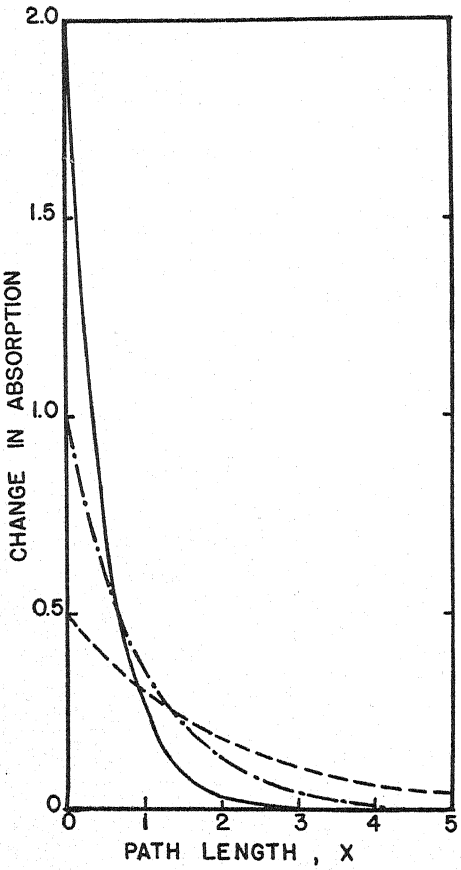


FIG. 16.—Change in energy absorbed with depth for three absorption coefficients corresponding to three different wave lengths of incident light:

$$\frac{dI}{dx}\,(\text{ordinates}) = -aI_0 e^{-ax}.$$

are colorless compounds. A reaction chain of considerable length undoubtedly exists between the initial step and the final control.

Reciprocity experiments and experiments involving irradiation at various times after the start of the dark period, which were conducted to determine the practicability of certain experimental routines, also contribute significant data concerning the nature of the photoperiodic reactions involved. These experiments indicate that the compound involved in the photochemical reaction either never diffuses from the chloroplasts or diffuses very slowly.

Summary

1. The purposes of this investigation were to obtain quantitative data on the photoreactions that prevent flowering of short-day plants, from which an action spectrum relating wave length to photoperiodic effectiveness of light could be derived, and to draw such inferences concerning the nature of the photoreactions as the action spectrum would permit.

2. Experiments designed to give the action spectrum made use of a specially designed prism spectrograph having a dispersion of 15 A. per cm. at 5000 A. At this wave length and with an effective slit width of 100 A. the energy was about 3000 ergs per sq. cm. per second with the slit illuminated by a carbon arc operated at 12 kw. input.

3. Plants investigated were soybean, *Soja max* (L.) Piper var. Biloxi, and cocklebur, *Xanthium saccharatum* Wallr. To facilitate irradiation, the foliar surface of the plants of each species at the beginning of an experiment was reduced to a single leaflet or leaf, respectively.

4. The experimental treatment was based on the fact that flowering of short-day plants receiving short photoperiods can be prevented by interrupting each of the accompanying long dark periods at or near the middle with a brief period of irradiation. In these experiments minimal energies required to prevent floral initiation were determined for many narrow regions of the spectrum.

5. The reciprocity law was tested and found to hold for the factors of time and intensity within the ranges used for dark-period interruption. Flowering response of both soybean and cocklebur depends on the total radiant energy used to interrupt the dark period.

6. Minimal energies necessary to prevent flowering depend upon the number of hours of darkness elapsing before the plants are irradiated but are constant over a 2-hour period beginning 30 minutes before the middle of the dark period.

7. Action spectra for soybean and cocklebur are similar in several respects. The limit of effectiveness of radiation for preventing flowering of both plants is near 7200 A., at the red end of the spectrum. Maximum effectiveness for both plants occurs over a broad region extending from about 6000 to 6800 A. A minimum of effectiveness of radiation for both plants occurs in the region of 4800 A., and effectiveness increases again at shorter wave lengths in the visible portion of the spectrum.

8. Ratios of energies required to prevent flowering in the regions of maximum and of minimum effectiveness were different for the two plants, being about 1:200 for cocklebur and 1:60 for soybean.

9. The possibility that chlorophyll may be the effective pigment in the photoperiodic reaction is examined.

Mrs. LAURA E. RAPPLEYE and Miss EMILY DUCHESNE gave valuable assistance with the various experiments.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BLAAUW, A. H. Die perzeption des lichtes. Rec. Trav. Bot. Néerl. 5:209–372. 1909.
2. BORTHWICK, H. A., and PARKER, M. W. Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. BOT. GAZ. 99:825–839. 1938.
3. ———, and ———. Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. BOT. GAZ. 101:806–817. 1940.
4. CAJLACHJAN, M. H. On the mechanism of photoperiodic reaction. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 1:89–93. 1936.
5. DU BUY, H. G., and WOODS, M. W. Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivations. II. Chemical evidence. Phytopath. 33:766–777. 1943.
6. EMERSON, ROBERT, and LEWIS, C. M. The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light. Amer. Jour. Bot. 30:165–178. 1943.
7. HAMNER, K. C., and BONNER, J. Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1938.
8. HOOVER, W. H. The dependence of carbon dioxide assimilation in a higher plant on wave length of radiation. Smithsonian Misc. Coll. 95 (21): 1–13. 3 plates. 1937.
9. JOHNSTON, E. S. Phototropic sensitivity in relation to wave length. Smithsonian Misc. Coll. 92 (11):1–17. 2 plates. 1934.
10. KATUNSKIJ, V. M. Dependency of photoperiodic reactions of plants on the spectral composition of light. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 15:509–512. 1937.
11. KLESHNIN, A. F. On the role of spectral composition of light in photoperiodic reaction. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 40:208–211. 1943.
12. KNOTT, J. E. Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. 31:152–154. 1934.
13. MOSKOV, B. S. The role of leaves in the photoperiodic response of plants. Bull. Appl. Bot., Genet., and Plant Breeding. Ser. A. (17):25–30. 1936.
14. PARKER, M. W. Environmental factors and their control in plant experiments. Soil Sci. 62:109–119. 1946.
15. PARKER, M. W.; HENDRICKS, S. B.; BORTHWICK,

H. A.; and SCULLY, N. J. Action spectrum for the photoperiodic control of floral initiation in Biloxi soybean. Science 102:152–155. 1945.
16. RAZUMOV, V. I. The significance of the qualitative composition of light in photoperiodical response. Bull. Appl. Bot., Gen., and Plant Breeding, III. Phys., Biochem., and Anat. Plants 3: 217–251. 1933.
17. WALD, GEORGE, and DU BUY, H. G. Pigments of the oat coleoptile. Sci. 84:247. 1936.
18. WITHROW, R. B., and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wave length of artificial light used to lengthen the daylight period. Plant Physiol. 11:225–249. 1936.
19. WITHROW, R. B., and BIEBEL, J. P. Photoperiodic response of certain long and short day plants to filtered radiation applied as a supplement to daylight. Plant Physiol. 11:807–819. 1936.
20. WITHROW, R. B., and WITHROW, ALICE P. The effect of various wave bands of supplementary radiation on the photoperiodic response of certain plants. Plant. Physiol. 15:609–624. 1940.

BREEDING BEHAVIOR AND CHROMOSOME NUMBERS IN PROGENIES FROM TWIN AND TRIPLET PLANTS OF POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

Many workers have observed that *Poa pratensis* L. develops a high proportion of polyembryonic seeds, and several reports have been published concerning the appearance of mature plants developed therefrom. There are few data, however, relative to the breeding behavior of the plants. It is the purpose of this paper to set forth additional observations on this problem as well as to correlate them with chromosome numbers.

Review of literature

VUKULOV (10) found that 5.6% of the total number of seeds of *P. pratensis* studied were polyembryonic. Among other congeners studied were *P. palustris* L. with 0.8%, *P. nemoralis* L. with 0.7%, *P. compressa* L. with 0.3%, and *P. trivialis* L. with 0.13% polyembryonic seeds. Excellent illustrations of twin and triplet seedlings accompanied the data.

The sizes of stomatal openings within each of five sets of twins of *P. pratensis* having unequal chromosome numbers were compared with those of two sets of twins with equal chromosome numbers (8). There appeared to be a positive correlation between chromosome numbers and the lengths of the stomata, but the number of comparisons possible was not sufficiently large to permit NISSEN to arrive at reliable conclusions.

ÅKERBERG (1) obtained an average of 2.9% polyembryonic seeds in *P. pratensis*. Apomictic types considered in other studies averaged 11.2%, sexual ones 3.4%, and 37 collections of diverse origin 9.8% polyembryonic seeds. Approximately 13% of thirty-two twin sets consisted of divergent members, and in another trial approximately 7% of two hundred and sixty-two plants (apparently one hundred and thirty-one sets) were dissimilar.

About one half of thirty-one sets of twin seedlings of *P. pratensis* examined

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, and the Wisconsin Agricultural Experiment Station, Madison. The statistical computations were made by personnel of the Statistical Laboratory of the College of Agriculture.

by SKOVSTED (9) had the diploid chromosome number, the remainder having deviating numbers. The seedlings of seven of the sets with equal numbers were considered to be of equal size, two of almost equal size, and seven others had pronounced differences. The seedlings of the fifteen other sets that had members with deviating chromosome numbers were classified as follows: three pairs were equal in size, three other pairs were almost equal in size, and nine pairs differed markedly within sets. Some of the latter sets were diploid-triploid. There did not appear to be any consistency as to which member was the more vigorous. One set was considered to be haploid-diploid, the haploid being the less vigorous as a seedling.

MÜNTZING (6) examined two hundred and seventy sets of *P. pratensis* twins. Eighteen plants were triploids, two were haploids, and five other deviating numbers were observed. He found that approximately 9% of all the plants had deviating chromosome numbers. He had concluded earlier (5) that usually the members of a twin pair have the same chromosome number but that sometimes one of the twins may be triploid to the diploid. The triploid plants were definitely more vigorous than their diploid mates. He suggested the hypothesis that in some of the Gramineae there may occur two or sometimes three macrospore mother cells. As a rule both of the embryo sacs derived from these macrospore mother cells are reduced, but sometimes one member may be diploid and the other haploid. Fertilization of the egg of each of these would consequently give rise to the diploid-triploid relationship. Likewise when haploid embryo sacs are present, the fertilization of the egg of one may stimulate the other to develop parthenogenetically so that haploid-diploid pairs would appear. Following further investigations (7) he reported that a triploid ($3n$) twin was usually less productive than its mate with the lower diploid ($2n$) chromosome number. It appeared that the triploid plant needed a longer time to reach maturity. Also, members with higher numbers had thicker and broader leaves than their corresponding twins with lower numbers. No difference could be found between plants in certain chemical characteristics. Pollen fertility was usually greater in the higher member of the set, and it seemed as though a change from the normal chromosome number to the triploid condition involved a change from a predominantly apomictic type of seed formation to a more or less pronounced degree of sexuality.

Several sets of twins and triplets were isolated by KIELLANDER (4) from an open-pollinated *P. pratensis* progeny ($2n = 72$) that had been completely uniform through three generations. Among these were two aberrant forms for the progeny, a twin with $2n = 40$, a triplet with $2n = 18$ that resembled *P. trivialis* ($2n = 14$). The $2n = 18$ triplet was partially fertile and produced offspring with the diploid number of chromosomes ranging from 15 to 19, the average number being 16.

Approximately 7% of the seed samples taken by BRITTINGHAM (3) from plants of 115 progenies were polyembryonic. The range in percentages varied from 0 to 31.6. The highest frequency of seeds with triplet embryos from a single plant was 4.3%. Sixty-nine twin sets and four triplet sets were grown to maturity. Approximately 17% of the sets were considered to be dissimilar, and it was concluded that the variability was significantly higher among plants derived from polyembryonic seeds than that among

plants derived from single-embryo seeds. Although this difference was noted, the nature of variation was considered essentially the same. Likewise, no association was found between plant type or manner of origin and the frequency of polyembryony in the species.

Material and methods

Two groups of progenies have been analyzed for the frequencies of aberrants, i.e., deviations from the predominant type. Twenty-four paired progenies (group I) from twins and two from triplets were established in the field in 1942 and subsequently studied in 1943. The parents of this group had been isolated by F. W. TINNEY.

Sixty-two sets of twins and four sets of triplets (group II) were isolated in March, 1942, from diverse lots of seed germinated in connection with other studies. The seedlings isolated cannot be taken as a random sample of Kentucky bluegrass twins, since an effort was made to isolate more dissimilar pairs with the consequence that numerous apparently identical seedling sets were not propagated. The plants of selected sets were separated and later space-planted into the field in May. These were classified as to their morphological similarity or dissimilarity during their first fruiting year. Individual panicles were harvested separately from each plant. Fifteen sets of twins and three sets of triplets were selected for further study. The basis for selection of these was morphological similarity or dissimilarity among the members of a given set. Derived progenies of twenty-four plants each were established in the field in 1944, and these were subsequently analyzed for their frequencies of aberrants during the summer of 1945.

Results

GROUP I

The data for the frequencies of aberrants among progenies are given in table 1. The chi-square test for independence shows that in three sets the percentage of aberrants in the progeny of one member of the pair is significantly different from that in the other. YATES' (11) correction for continuity was used, since there was only one degree of freedom in each comparison. The frequency of aberrant plants in line 251 differed at the 1% level and in lines 275 and 279 at the 5% point. No difference was ascertainable among the triplet progenies.

GROUP II

THE PARENT PLANTS.—The original plant isolations from which progenies were developed were analyzed for vigor, aggressiveness, plant height, texture, and other similar characters in June, 1943. Forty-six per cent of the twins were considered identical. Fifteen of the thirty-one sets of twins isolated from seed lots producing more than one set of twin seedlings were classified as dissimilar and sixteen as similar. At least one individual of the respective set was dissimilar in two of the four sets of triplets.

SEEDLINGS FROM TWINS AND TRIPLETS.—The germinating seed lots from the different pairs of group II parents were compared, relative to the number and vigor of the seedlings produced, during the seedling stage. Results are summarized in table 2.

The seed of one plant of each of two twin sets was completely sterile, no seedlings developing from either plant. That from their mates produced many and intermediate numbers of seedlings, respectively.

TABLE 1

NUMBERS OF PLANTS AND PERCENTAGES OF ABERRANTS IN PROGENIES FROM TWINS AND TRIPLETS OF POA PRATENSIS

Progeny no.	*a*-Progeny*		*b*-Progeny*		*c*-Progeny*	
	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)
237	24	4.2	24	0.0		
239	24	0.0	24	0.0		
241	24	4.2	24	4.2		
243	24	4.2	24	0.0		
245	24	4.2	24	4.2		
247	24	0.0	24	8.3		
249	24	8.3	24	4.2		
251	24	4.2	24	50.0		
253	24	4.2	24	4.2		
255	24	4.2	24	12.5		
257	24	4.2	24	0.0		
261	24	4.2	24	4.2		
263	24	0.0	24	0.0		
265	24	0.0	24	0.0		
267	24	8.3	24	4.2		
269	24	0.0	24	4.2		
273	24	8.3	24	4.2		
275	24	0.0	24	20.0		
277	24	4.2	24	8.3		
279	24	0.0	24	16.7		
281	24	8.3	24	16.7		
285	24	33.3	24	33.3		
287	24	4.2	24	8.3		
289	8	0.0	5	0.0		
291	15	0.0	24	0.0	24	0.0
294	24	4.2	24	0.0	24	8.3

* *a*, *b*, and *c* indicate progenies from different members of twin and triplet sets.

TABLE 2

RELATIVE NUMBER AND VIGOR OF SEEDLINGS FROM SINGLE PANICLES OF TWIN PLANTS OF POA PRATENSIS

No. of seedlings per set				Vigor of seedlings per set			
b-Plant	*a*-plant			*b*-Plant	*a*-plant		
	Many	Intermediate	Few		Good	Fair	Poor
Many	4(*4*)/8*	7(*6*)/13	5(*2*)/7	Good	5(*6*)/11	11(*7*)/18	0(*3*)/3
Intermediate		4(*9*)/13	5(*3*)/8	Fair		6(*9*)/15	6(*3*)/9
Few			5(*6*)/11	Poor			2(*2*)/4

* The first figure is for comparisons of similar plants of sets of twins; the italicized figure (in parentheses) is of dissimilar members of sets. The totals of each comparison in each class are given following the slanting line.

Significant differences were not found between the number of seedlings developed from the different sets. Approximately half of the total seed lots germinated similarly, and the vigor of the seedlings was essentially the same.

plant type or vigor. There is no significant difference between the germinability of seed of either type of average or better vigor. However, there is a tendency for nonvigorous plants to produce inferior numbers of viable seeds

TABLE 3

VIGOR AND SIMILARITY OF MEMBERS OF TWIN AND TRIPLET SETS OF POA PRATENSIS, SIMILARITY OF PLANTS OF THEIR PROGENIES, GERMINATION RECORDS, AND NUMBER OF PLANTS AND PERCENTAGE OF ABERRANTS IN PROGENIES

No.	Vigor of plants			Similarity of		Seedling*						*a*-Progeny†		*b*-Progeny†		*c*-Progeny†	
						Av. no.			Vigor								
	a	*b*	*c*	Sets‡	Progenies‡	*a*	*b*	*c*	*a*	*b*	*c*	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)
36-1...	P	P		−	−	F	F		P	P		17	41.2	18	72.2		
252-1...	G	G		+	+	M	F		F	P		20	10.0	23	13.0		
252-2...	G	G		−	−	I	I		F	F		23	30.4	23	43.5		
252-4...	F	F		+	+	F	VF		F	P		22	31.8	21	23.8		
292-1...	G	G		+	+	I	I		G	F		22	4.5	21	4.8		
292-2...	G	G		+	+	F	F		F	F		20	10.0	20	25.0		
358-1...	F	P		−		M	o		P	−		23	52.2	Sterile			
397-1...	G	G		+	+	F	I		F	F		21	23.8	20	20.0		
397-3...	G	P		−		I	o		F	−		20	25.0	Sterile			
540-1...	G	F		−	−	M	I		G	P		22	27.3	20	100.0		
353-1...	G	F		−	−	F	F		P	P		23	4.3	22	4.5		
587-3...	P	P		+	+	F	F		P	P		21	14.3	27	13.6		
587-4...	F	G		−	−	M	M		F	G		18	33.3	22	40.9		
652-1...	F	P		−	−	I	I		F	P		22	100.0	21	66.6		
652-2...	G	F		−	−	I	I		F	F		22	36.4	21	23.8		
26-1...	F	F	P	−	−	F	M	F	P	F	P	18	18.7	23	17.4	23	4.3
34-1...	F	F	F	+	+	I	I	F	F	F	F	14	21.4	20	30.0	16	37.5
599-1...	G	G	G	−	−	F	I	F	F	F	F	21	9.5	22	9.1	20	40.0

* Average number and vigor of seedlings: M, many; I, intermediate; F, few; G, good; F, fair; and P, poor vigor.
† *a*, *b*, and *c* indicate progenies from different members of twin and triplet sets.
‡ Plus (+) symbols indicate similarity; minus (−) symbols dissimilarity.

The data for germination of seed lots from three sets of triplets are included in table 3. Many seedlings resulted from the seed lots from all members of the fourth set. Those from two plants were of good vigor, whereas those from the third were only fair in this respect.

A comparison (table 3) of germination records of plants of similar sets of twins with those of dissimilar sets shows that the number and the vigor of seedlings are characteristics generally unrelated to that develop into seedlings of low vigor. The data for sets of triplets are not sufficiently extensive to permit generalization.

PROGENIES.—Data for the second set of progenies from twin and triplet plants of Kentucky bluegrass are given in table 3. Results were analyzed similarly to those for the 1942 progenies. The chi-square test for independence indicated that the frequency of aberrants in progenies from three sets differed significantly.

These include sets 540-1, 599-1, and 652-1. Inspection of these data also indicates that the predominant plant types of any given set of progenies may be similar or different, a fact that a statistical test of this type cannot be used to determine. Likewise, the two progenies grown from the mates of sterile plants showed that one of these individuals was predominantly apomictic and the other intermediate or tending slightly toward the sexual mode of reproduction.

CHROMOSOME NUMBER.—Plants of six of the progenies from sets of twins and two of the progenies from triplets were examined for their chromosome numbers. The chromosome number reported for the predominant type is, in all cases, based upon approximations in one or two members of a progeny and a detailed analysis and check of one plant. Two or more plants of each progeny were examined. The chromosome numbers of two additional sets of twins were examined, these being the sets of which the seeds of one individual were sterile. Aceto-carmine smear preparations were used, and, with one exception (that at diplotene), diakinesis figures were studied for the determinations. It may be seen from figures given here, and from those of previously published papers, that the chromosomes of *P. pratensis* are small, often numerous, and have strong tendencies to bunch. The chromosome counts are summarized in table 4. Those of the higher numbers, particularly, should be considered to be only approximations of the actual number of a given biotype or line.

TABLE 4

APPROXIMATE CHROMOSOME NUMBERS IN PLANTS ISOLATED, OR IN PLANTS OF PROGENIES FROM TWINS AND TRIPLETS OF POA PRATENSIS*

Twin or triplet set number	*a*-progeny	*b*-progeny	*c*-progeny
358-1.....	56†	28†	
652-1.....	56	56	
587-3.....	56	57	
252-4.....	57 (80)‡	59 (98)‡	
587-4.....	86	58	
397-3.....	58†	117†	
540-1.....	84	56	
599-1.....	84	84	114 (86)‡
34-1.....	110	108	109

* Numbers calculated from meiotic complements observed approximately at diakinesis.

† Counts made upon twin plants isolated, of which one member of set proved to be sterile.

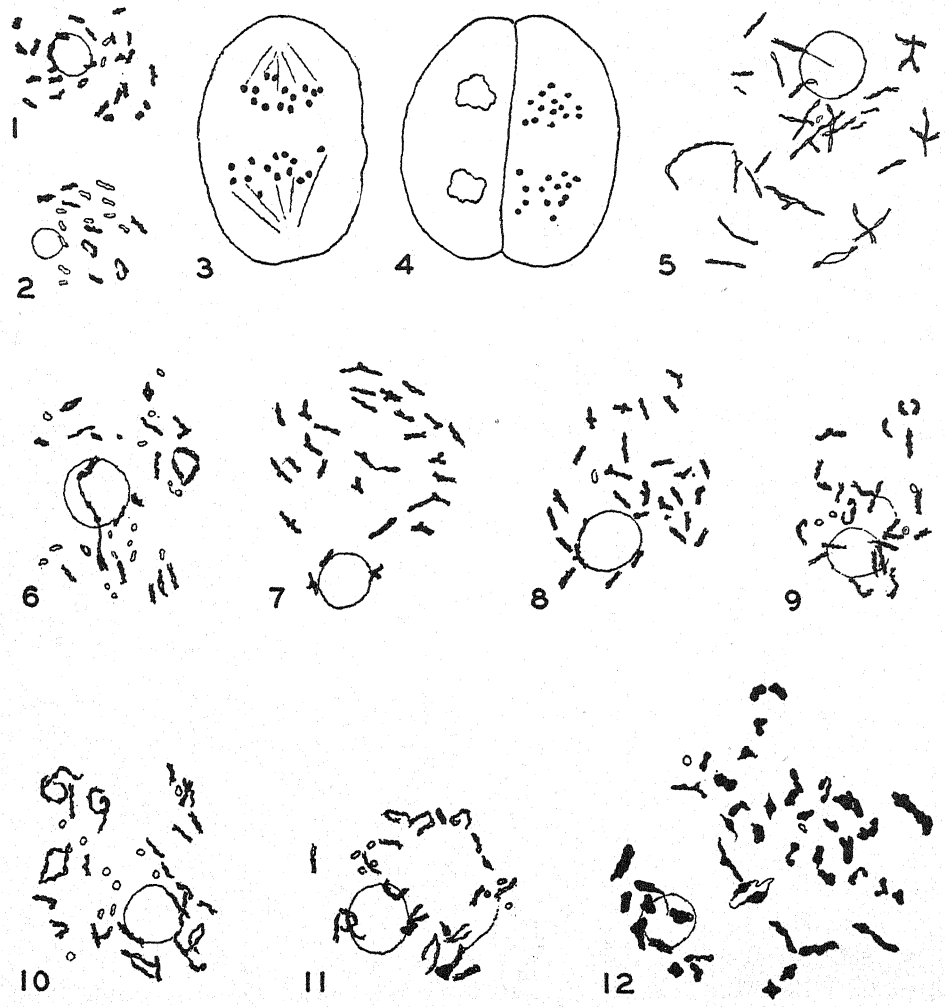
‡ Numbers in parentheses are of aberrants in progenies.

SEXUAL COMBINATIONS

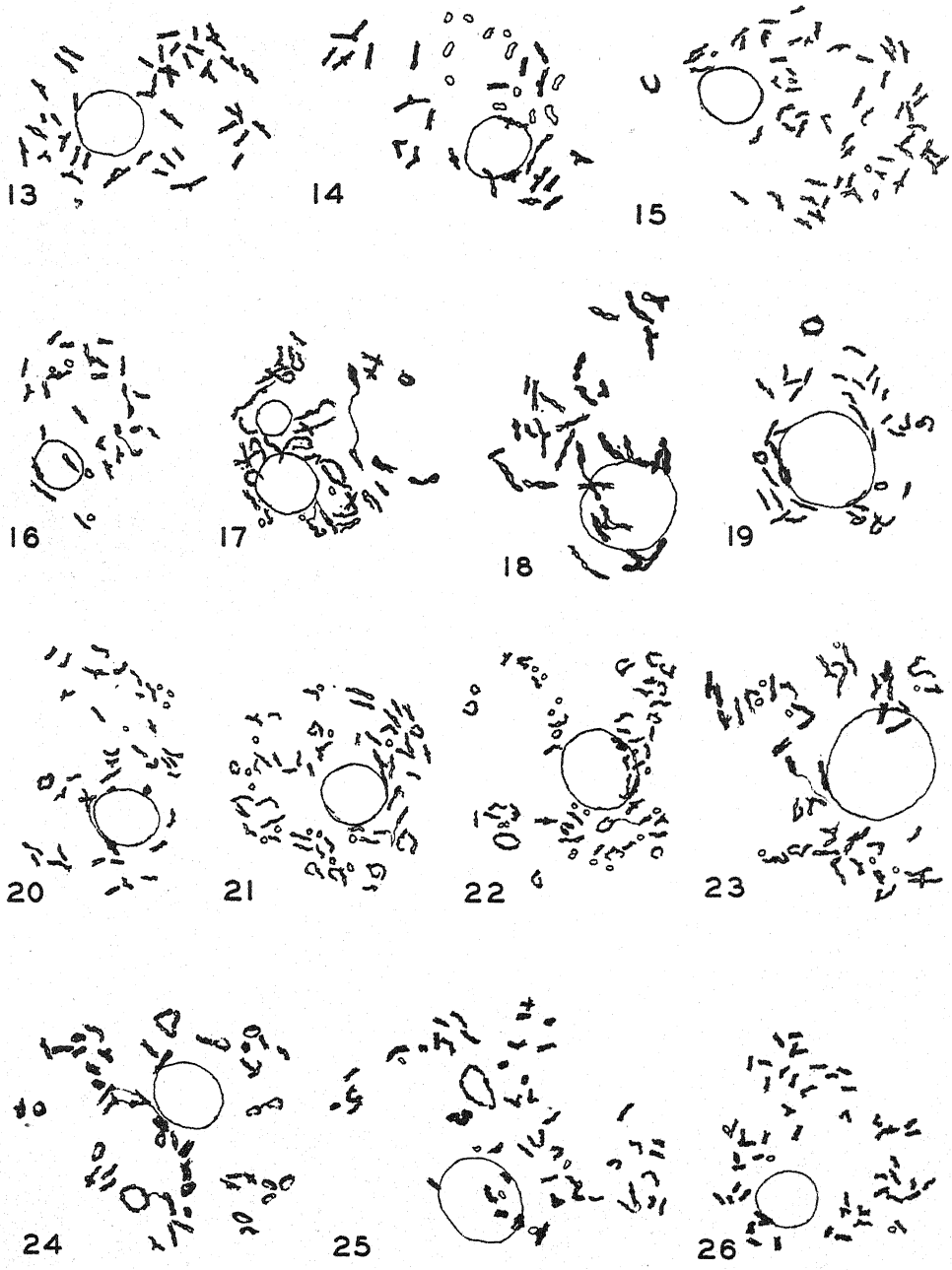
Two sets of progenies from twins of this category were examined. Members of the first pair (358-1, table 3) to be considered were very different in their morphological characteristics. One plant (*a*) was of fair vigor, 15 inches high, with leaves of medium length, and generally open panicles (fig. 27). The second plant (*b*) was very weak but survived the first winter after it was transplanted into the field. It continued to be weak; at flowering time the following summer, it reached a maximum height of 6 inches, had short leaves, and developed three short, compact panicles. One panicle was left to mature seed and the other two were fixed for cytological examination. This plant failed to survive the second winter. The *a*-plant (meiotic complement $\pm 27_{II} + 2_I$, fig. 1) was fertile, and the progeny of this plant contained approximately 52% aberrants. The *b*-plant was completely sterile. Its anthers were partially defective, but sufficient microspore mother cells developed so that at least some data were obtained. $8_{II} + 12_I$ (fig. 2) were usually present at diakinesis. Occasionally others had 6, 7, and 9 bivalents, with corresponding deviations of univalents. Fourteen chromosomes pass to

each pole at anaphase (figs. 3, 4). A chromosome fragment is sometimes present at this stage. Comparative data for other stages are given in table 5. Chromosome bridges and, in at least one case, a dicentric bridge with an acentric fragment were present. Additional observations concerning macrosporogenesis in the plant are given in the companion paper.

The data suggest origins of one or two methods of formation of twins: (1) the stimulation of a synergid of a reduced embryo sac by the act of fertilization of the contiguous egg or (2) the stimulation of the egg of one embryo sac of an ovule



FIGS. 1–12.—Meiotic complements in plants of *Poa pratensis:* Fig. 1, 358-1*a*. $27_{II}+2_{I}$. Fig. 2, 358-1*b*. $8_{II}+10_{I}$. Fig. 3, 358-1*b*. Anaphase$_{I}$. Fig. 4, 358-1*b*. Anaphase$_{II}$. Fig. 5, 652-1*a*- plant 3. 28_{II}. Fig. 6, 652-1*b*- plant 3. $2_{III}+16_{II}+17_{I}$ (?). Fig. 7, 587-3*a*- plant 3. 28_{II}. Fig. 8, 587-3*b*- plant 3. $28_{II}+1_{I}$. Fig. 9, 252-4*a*- plant 3. $27_{II}+3_{I}$. Fig. 10, 252-4*a*- plant 8. $1_{IV}+3_{III}+23_{II}+10_{I}$. Fig. 11, 252-4*b*- plant 5. $1_{III}+23_{II}+7_{I}$. Fig. 12, 252-4*b*- plant 2. $55_{II}+2_{I}$.

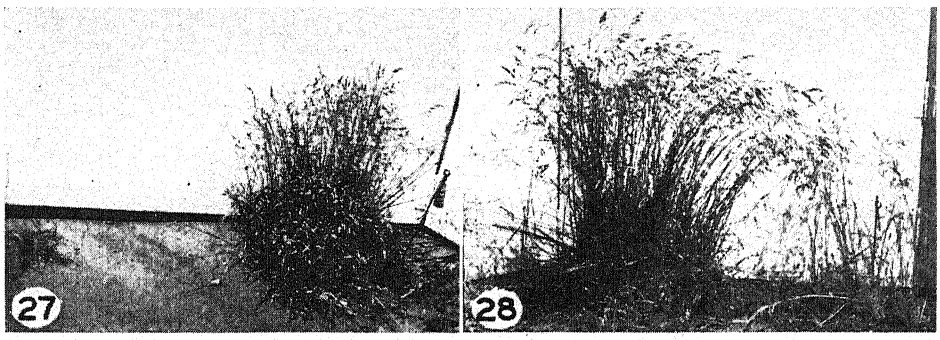


FIGS. 13–26.—Meiotic complements in plants of *Poa pratensis:* Fig. 13, 587-4*a*- plant 3. 43_{II}. Fig. 14, 587-4*b*- plant 4. $23_{II}+12_{I}$. Fig. 15, 397-3*b*. $57_{II}+3_{I}$. Fig. 16, 397-3*a*. $1_{III}+24_{II}+4_{I}$. Fig. 17, 540-1*a*- plant 3. $2_{III}+36_{II}$. Fig. 18, 540-1*b*- plant 6. 28_{II}. Fig. 19, 599-1*a*- plant 4. 42_{II}. Fig. 20, 599-1*b*- plant 6. 42_{II}. Fig. 21, 599-1*c*- plant 5. $1_{III}+48_{II}+13_{I}$. Fig. 22, 599-1*c*- plant 6. $1_{IV}+1_{III}+42_{II}+16_{I}$. Fig. 23, 599-1*c*- plant 14. $39_{II}+8_{I}$. Fig. 24, 34-1*a*- plant 5. $1_{VI}+1_{IV}+1_{III}+42_{II}$. Fig. 25, 34-1*b*- plant 9. $1_{III}+49_{II}+4_{I}$. Fig. 26, 34-1*c*- plant 7. $1_{III}+50_{II}+3_{I}$.

as a result of fertilization of the egg of the second embryo sac.

The second set of twins (652-1) was also made up of quite dissimilar members (fig. 28). One plant (*a*) was vigorous, somewhat aggressive, medium in texture, and at maturity reached a height of 29 inches. The second plant was of poor vigor, nonaggressive, medium texture, and, at maturity, was 19 inches tall. All members of the progeny developed from the *a*-plant differed from one another in morphology. Representatives of this progeny contained approximately 28_{II} at diakinesis (fig. 5). Of the progeny from the *b*-plant, 67% were classified as morphologically dissimilar. Generally they were inferior to those of the sister *a*-progeny in vigor, leafiness, and height. Members of this progeny averaged about $2_{III} + 16_{II} + 17_{I}$ at diakinesis, with multiple associations of three chromosomes being common (fig. 6).

It would appear that this set of twins arose through the fertilization of an egg and a synergid of one embryo sac or, possibly, through the fertilization of the eggs of two different embryo sacs in the same nucellus. Noncompatibility as evidenced by frequent unpaired chromosomes of the *b*-progeny plants may have been sufficiently effective to reduce the general vigor of the plants of the progeny.



FIGS. 27–28.—Mature twin plants of *Poa pratensis:* Fig. 27, *left*, 358-1*b*; *right*, 358-1*a*. Fig. 28, *left*, 652-1*a*; *right*, 652-1*b*.

TABLE 5

AVERAGE NUMBER OF LAGGARDS PER SPOROCYTE AT METAPHASE I, ANAPHASE I, TELOPHASE I, DIAD, AND MICRONUCLEI PER QUARTET IN HAPLOID-DIPLOID POA PRATENSIS TWIN PLANTS

Plant	*n*	Metaphase I	Anaphase I	Telophase I	Diad	Quartet
358*a*..	28	1.88	2.28	1.00	0.84	1.32
358*b*..	14(+*f*)	1.00	1.00	0.80	0.20	0.44

PREDOMINANTLY APOMICTIC COMBINATIONS

SIMILAR SETS OF TWINS.—Two sets of twin progenies wherein the predominant plant type was similar were examined for chromosome numbers. Members of set 587-3 were essentially equal in frequencies of aberrants, these being 14.3 and 13.6%, respectively. Generally plants from the *a*-parent had 28 bivalents whereas those from the *b*-parent had $28_{II} + I_{I}$ (figs. 7, 8, 29, 30) at meiosis.

Plants of the predominant type from the second set of twins (252-4), with approximately 32 and 24% aberrants, had

$\pm 27_{II} + 3_I$ (fig. 9) and $\pm 1_{III} + 23_{II} + 7_I$ (fig. 11),[2] respectively. One aberrant from the *a*-plant had $\pm 1_{IV} + 3_{III} + 23_{II} + 10_I$ (fig. 10), or an equivalent of 35 bivalents at meiosis. An aberrant plant of the mate or *b*-progeny had about $55_{II} + 2_I$, or the equivalent of 56 bivalents (fig. 12).

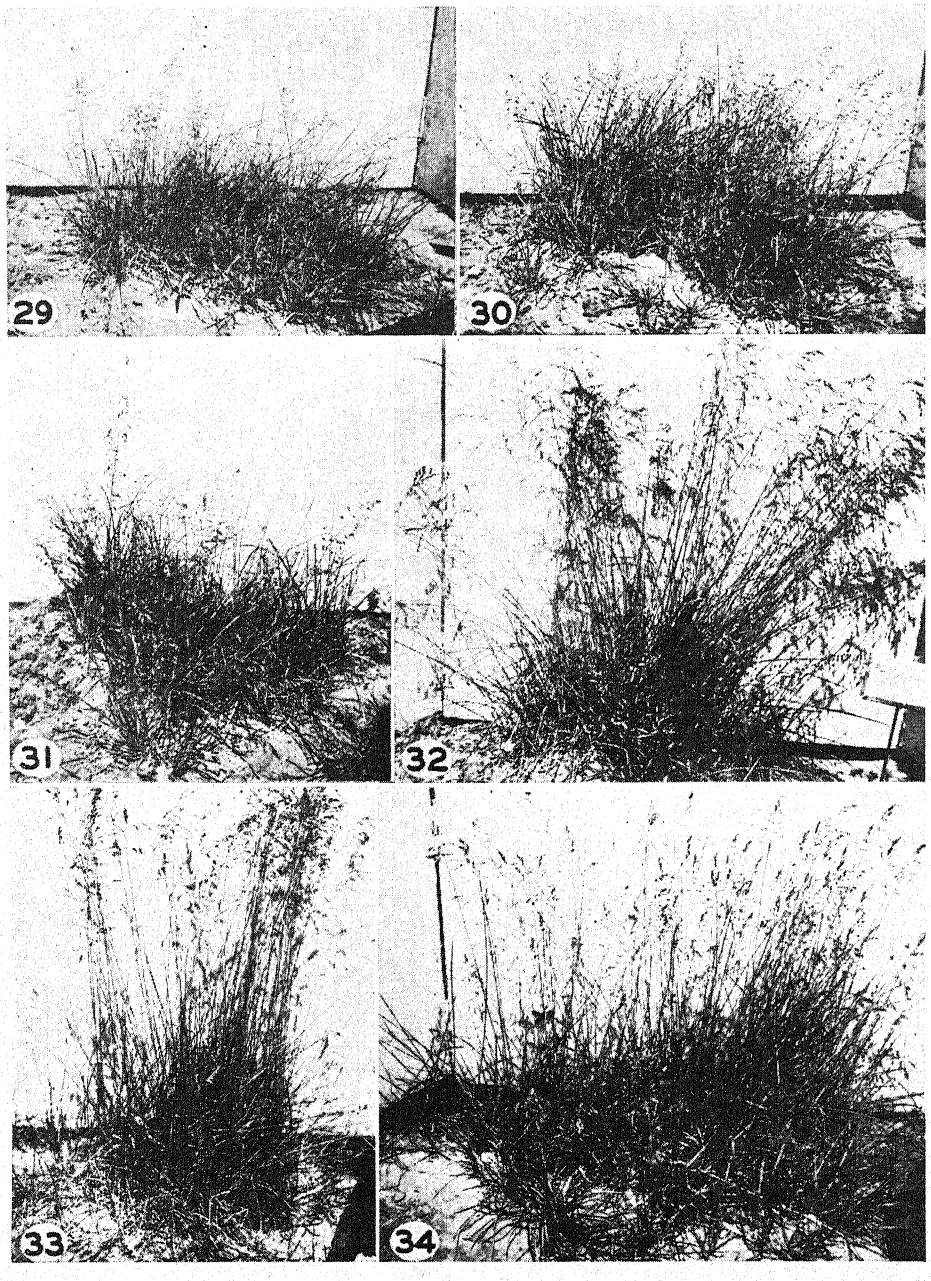
These observations suggest that twin embryos developed in ovules of the parents of 587-3 and 252-4 without fertilization of the eggs. Both twin sets developed progenies of low and essentially equal frequencies of aberrants. In addition, however, seedlings derived following fertilization were represented in both of these progenies. The aberrant in the *a*-progeny (252-4*a*- plant 8) apparently arose through the fertilization of the egg cell of a somatic aposporous embryo sac with a male gamete carrying approximately the reduced number of chromosomes ($n = 28$). The aberrant of the *b*-progeny examined (252-4*b*- plant 2), with $\pm 55_{II} + 2_I$, is somewhat more difficult to explain. The observations suggest either fertilization by an unreduced gamete or a somatic doubling of the dividing zygote nucleus.

Based upon progeny behavior, four other sets of twins were probably of this type (252-1, 292-1, 292-2, and 397-1).

DISSIMILAR SETS OF TWINS.—Members of two sets of progenies, wherein the predominant type in a given progeny was unlike that of their mates, were examined. The members of the sets of twins 587-4 were markedly dissimilar, differing in growth habit and aggressiveness, texture or coarseness of both culms and leaves, general vigor, and plant height (figs. 31, 32). Although progenies developed from twin plants did not differ significantly in their frequencies of aberrants (40.9 and 33.3%), the morphological characteristics of the predominant type of two progenies differed greatly. Members of the *b*-progeny had about $23_{II} + 12_I$ (fig. 14), whereas those of the other had approximately 43_{II} (fig. 13). This is interpreted to mean that the ovule of the plant wherein these twins developed contained either two aposporous embryo sacs, of which the egg of each formed a functional embryo, or that it contained an embryo sac that also arose through somatic apospory. One of the synergids could have developed because of the stimulus given it by the fertilization of the egg. In either case it appears that a second egg or a synergid developed parthenogenetically because of the stimulus set up by the fertilization of the other functional member in the ovule. Although the end results would be the same, the last-mentioned alternative seems the more plausible, based upon the relative frequencies of multiple embryo sacs observed.

The observations on the second set (397-3) are somewhat more difficult to interpret. In this set the two members of the twin set differed markedly in vigor, aggressiveness, texture, and plant height, the member designated as *b* being, in all characters mentioned, inferior to those of the mate (*a*). The relative heights were 10 and 23 inches, and the chromosome numbers about $57_{II} + 3_I$ (fig. 15) and $\pm 1_{III} + 24_{II} + 4_I$ (fig. 16), respectively. Plant *b* proved to be sterile as determined by seed germination for subsequent progeny tests; plant *a* produced numerous functional seeds and the mature progeny therefrom had 25% aberrant plants. MÜNTZING (5) suggested that the occurrence of tetraploids among twins most probably implies somatic doubling after fertilization or possibly

[2] It was not possible in the material examined to determine whether the multiple associations of chromosomes were due to homologies or to catenations. They are therefore considered as multiple associations of either or both types.



FIGS. 29–34.—Members of progenies from twins in *Poa pratensis:* Fig. 29, 587-3*a*- plant 10. Fig. 30, 587-3*b*- plant 14. Fig. 31, 587-4*a*- plant 20. Fig. 32, 587-4*b*- plant 14. Fig. 33, 652-2*a*- plant 2. Fig. 34, 652-2*b*- plant 4.

doubling at the beginning of embryo development if this has started parthenogenetically. Other possible explanations for this condition should be mentioned. Only one of these appears at all likely to occur. The ovule from which this polyembryonic seed developed perhaps contained two embryo sacs that had arisen by somatic apospory. The maternal tissue in this case probably contained about 56 chromosomes. One egg of these embryo sacs was stimulated to develop parthenogenetically and eventually resulted in the fertile plant. The second egg may have been fertilized either by an unreduced gamete or by a high polyploid gamete carrying approximately the same chromosome number as the egg. Although pairing was evident, subsequent interference may have resulted in complete sterility. It is quite unlikely that the combination represents a true haploid-diploid relationship of embryos developed from a fertilized egg and a synergid of a single embryo sac. Judging from other experiences the haploid would either be sterile or, if fertile, the progeny therefrom would show considerable segregation. The opposite is true in this case. It is likely that the first of the alternatives, that suggested by MÜNTZING, is the most plausible explanation of the case in question. However, the second alternative is possible but is not likely to be of common occurrence. Restitution nuclei were not observed, or so recognized, in any abundance, and very high polyploids were comparatively uncommon in the material examined.

The progeny behavior of three other sets of twins (252-2, 353-1, and 652-2 [figs. 33, 34]) suggests similarity to 587-4. Members of two of these sets, however, tend to produce fairly high proportions of aberrant offspring.

MIXED SEXUAL AND APOMICTIC COMBINATIONS

The progeny of one member of a set of twins (540-1) was predominantly uniform (27.3% aberrants), whereas the progeny from its mate was extremely diverse, no two plants being entirely alike. In this case plants of the predominant type of the former contained approximately $2_{III} + 36_{II}$ (fig. 17), whereas plants of the sister progeny had 28_{II} (fig. 18). Comparatively few plants of the species are either entirely apomictic or entirely sexual in their modes of reproduction, the majority of plants being about 20% sexual. Plants of intermediate tendencies are relatively common. It has been observed that there sometimes occurs in the same ovarian primordium a functional macrospore mother cell and a densely cytoplasmic nucellar cell similar to those found in apomictically reproducing forms. Usually one of these, or its descendants, will fail to survive, particularly so when one or the other is of advanced development. When the development is synchronous or essentially so, embryos may form in both embryo sacs. The difference between the pair of twins in question (540-1), and their progenies, seems to be explained best in this manner. If the egg of the embryo sac having the gametophytic chromosome number is fertilized, a progeny of diploid plants would result. Because of the apparent heterozygosity of plants of *P. pratensis*, a highly or even totally diverse progeny would be expected. This is based upon the assumption that all fertile florets of a given plant contained reduced gametophytes, the eggs of which were fertilized. The egg cell of the somatic aposporous embryo sac might be stimulated to develop either a diploid embryo parthenogenetically or, if fertilization by a re-

duced gamete occurs, an embryo of triploid constitution.

The breeding behavior of one set of twins (36-1) does not appear to fall definitely into any one single class. One plant of this set produced 41% aberrants compared with 72% for its mate. It appears evident that both these plants carry intermediate to strong tendencies toward sexuality, and it is equally evident that, though these tendencies are strong, neither plant is entirely sexual or strongly apomictic. In default of any better classification of the set, it is included here.

Triplets

Similar sets.—The predominant plant type was similar in all three progenies from one set of triplets (34-1). The progenies contained 21.4, 30.0, and 37.5% aberrants, respectively, for *a*, *b*, and *c*. Representative plants of the predominant type of each of these progenies contained about the following chromosome complements: $1_{VI} + 1_{IV} + 1_{III} + 42_{II}$ (fig. 24), $1_{III} + 49_{II} + 4_{I}$ (fig. 25), and $1_{III} + 50_{II} + 3_{I}$ (fig. 26), respectively. These are interpreted to indicate the development of the triplet set from the eggs of three aposporous embryo sacs in the same ovule; or the development of embryos from all members of the egg apparatus of a single sac; or an intermediate combination of two embryos arising from members of one embryo sac and one from those of a second embryo sac. In any event, it appears unlikely that fertilization of the egg of any one of these took place. Had such fertilization been effected, there would perhaps have been a phenotypic as well as a cytologic difference expressed in the progeny from one member. There is evidence that fertilization is not necessary for embryo development in apomictic biotypes of *P. pratensis* (2).

Dissimilar sets.—Progenies from two members of a set (599-1) of triplets were predominantly uniform and morphologically similar. A few of the plants (9–10%) of each of these progenies were aberrant. Sixty per cent of the plants from the third triplet were generally more vigorous and differed morphologically from those of the sister members of the set. Characteristically, plants of the two similar members of the set had chromosome constitutions approximating 42_{II} (figs. 19, 20) compared with equivalents of 57_{II} to 59_{II} (figs. 21, 22) in individuals of the third progeny. This combination is essentially similar to several cases previously indicated for twins. The mode of origin of this set of triplets is a matter of conjecture. It is most probable that they arose from at least two embryo sacs, the only basis for this surmise being, however, that in no instance in this or in earlier reported studies have all members of the egg apparatus of a single embryo sac been seen to develop. Plant 14, one of the aberrants of the progeny (599-1) with 57–59 bivalents, had approximately 42_{II} (fig. 23). The most logical explanation of this plant would appear to be the fertilization of a reduced egg (56_{I}) with a male gamete carrying 28 chromosomes. This would result in a plant with $2n = \pm 84$, which corresponds with the count made.

Discussion

Data presented in this paper clarify, in part at least, some of the problems that have arisen in connection with the chromosome numbers and the highly variable gross morphology of the different biotypes of *P. pratensis*. The most recent tabulation (2) of somatic chromosome

numbers reported for the species indicates a range of from 24 to ±124, exclusive of calculated complements. In the same paper ÅKERBERG reported that a biotype from Germany had $2n = \pm 130$. Twenty-five of the numbers were euploid, the remaining 127 being aneuploid. Graphically the distribution of the more definite chromosomal counts is a skewed trimodal curve with apices in the 49–56, 63–70, and 84–91 chromosome-number classes. These apices each represent 38, 29, and 17 determinations, respectively.

The combination of both sexual and apomictic modes of reproduction, coupled with free interpollination of biotypes and apparently weak genetic barriers, with all their possible interactions, appears to be a sufficient explanation for the chromosome and morphological diversity of *P. pratensis*.

Haploid plants of this species have not been extensively studied. Most reports concern chromosome number only. The single plant of this type ($n = 14$) considered here was very weak. Subsequently the plant proved to be sterile. This is not always the case. MÜNTZING (7) found a haploid ($n = 36$) that produced a few seeds after self-pollination. Twenty-one of twenty-nine resulting plants examined had 36 chromosomes, one each had 29, 34, 37, 49, and 52, and three had 35. Plants from seed of florets allowed access to open-pollination generally had from 32 to 58 chromosomes. An analogous case was also presented for *P. alpina*. A somewhat similar instance has been reported by KIELLANDER (4) for open-pollinated *P. pratensis*. These examples are sufficiently illustrative of the diversity that may arise in progenies from plants with low chromosome numbers.

Aside from the data given in this paper, the writer is aware of chromosome counts only for the exceptionally high polyploids of the species. The reduced vigor and the accompanying sterility of the plant reported here suggest that this approaches or has reached the chromosomal limits wherein fertility might be expected to exist. Examination of the germination records of other plants (252-4*b* and 599-1*c*) with high chromosome numbers tends to support this opinion. In both cases relatively few seedlings developed from the seed of individual panicles. The seedlings of 599-1*c* were classified as of fair vigor and those of 252-4*b* as poor.

The synthesis of new chromosome and genetic combinations between the above-indicated extremes can apparently be accomplished more or less successfully without serious detriment to the fertility of the resulting new combination. Apomixis, accompanied by sexual reproduction in various proportions of florets, thus affords in some measure a mechanism for maintaining an unstable genetic and chromosomal condition in the biotypes. The end result of such a condition of reduction and accumulation of genetic factors and chromosomes can only be a multiplicity of forms and considerable diversity in breeding behavior.

Summary

Two groups of twin and triplet sets are considered. Twenty-four pairs of twins and two sets of triplets (group I) were grown to maturity from seedlings. The frequency of aberrants in progenies from three sets of twins was found to differ significantly. No such difference occurred between the progenies from the other twenty-one sets of twins or between those from the two sets of triplets.

Forty-six per cent of a nonrandom

population (group II) of twin and triplet sets differed in morphological characteristics. No significant differences were found between the number and the vigor of seedlings from members of multiple sets. Likewise, no difference occurred between the same characters for identical compared with dissimilar sets.

The frequency of aberrant plants was significantly different in progenies from two of the twin and one of the triplet sets. The gross morphology of the predominant plant type in progenies from seven twin and two triplet sets was greatly different.

It was concluded from the breeding behavior and the chromosome number in plants of progenies, and from two sets of twins, that multiple embryos had arisen in the following manners: (1) from the egg and a synergid of an embryo sac carrying the reduced chromosome number, one member of the egg apparatus developing parthenogenetically because of the stimulative effect of fertilization of the other; (2) from two embryo sacs in which the eggs (or an egg and a synergid) carrying the gametophytic chromosome number had been fertilized; (3) from the unfertilized eggs, or from an egg and a synergid, of embryo sacs that arose by somatic apospory; (4) from eggs (or the egg and a synergid of one sac) of embryo sacs developed through somatic apospory, one forming a diploid embryo and the other a tetraploid by somatic doubling of the dividing zygote; (5) from two egg cells or from an egg and synergid that arose by somatic apospory, one of which was fertilized, the other developing parthenogenetically; and (6) from the fertilized egg derived from a reduced macrospore and from an egg of an aposporous embryo sac.

The observations are discussed in relation to known breeding behavior of biotypes of the species and to the morphological and chromosomal variation in naturally occurring populations of the species.

The writer gratefully acknowledges the suggestions made by colleagues during the progress of this study.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ÅKERBERG, E. Apomictic and sexual seed formation in *Poa pratensis*. Hereditas **25**:359–370. 1939.
2. ———. Further studies on the embryo and endosperm development in *Poa pratensis*. Hereditas **29**:199–210. 1943.
3. BRITTINGHAM, W. H. Type of seed formation as indicated by the nature and extent of variation in Kentucky bluegrass, and its practical implications. Jour. Agr. Res. **67**:225–264. 1942.
4. KIELLANDER, C. L. A subhaploid *Poa pratensis* L. with 18 chromosomes and its progeny. Svensk. Bot. Tidskr. **36**:200–220. 1942.
5. MÜNTZING, A. Polyploidy from twin seedlings. Cytologia. Fyjii Jubilee Vol. 1937.
6. ———. Note on heteroploid twin plants of eleven genera. Hereditas **24**:487–491. 1938.
7. ———. Further studies in apomixis and sexuality in *Poa*. Hereditas **26**:115–190. 1940.
8. NISSEN, Ö. Spalteåpningenes störrelse hos tvillingplanter med. ulike Kromosontall. Bot. Not. Pp. 28–34. 1937. (Engl. summary.)
9. SKOVSTED, A. Cytological studies in twin plants. Comptes Rendus des travaux du Laboratorie Carlsberg Ser. Physiologique **22**:427–446. 1930.
10. VUKULOV, V. A. Ueber die Polyembryonie bei Rispengras. Sbornik. ceskaoslov. Akademie zemedelska **4**. 1929. (Engl. summary, pp. 217–218.)
11. YATES, F. Contingency tables involving small numbers and the X^2 test. Jour. Roy. Stat. Soc., Suppl. i, no. 2. Pp. 217–235. 1934.

THE ORIGIN OF MULTIPLE MACROGAMETOPHYTES IN POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

There has been some divergence of opinion concerning the origin of multiple embryos in *Poa pratensis* L. This paper will consider briefly the scope of the problem and indicate the conclusions arrived at by other workers. Additional data have been obtained and are presented so that integrated conclusions may be reached. Confirmatory evidence from breeding behavior and chromosome numbers in plants from twins and triplets has been obtained and is presented for most of the types of gametophytes (and subsequent embryo development) observed or postulated from these cytological studies (10).

Review of literature

Individual seeds of *P. pratensis* sometimes contain from one to three embryos (14). ZIMMERMAN accredits a Dr. HEINRICH with previously observing a large number of twin embryos and, in one case, three distinct seedlings developing from a single seed. ZINN (15) also noted polyembryonic seeds in *P. pratensis*, *P. nemoralis* L., and *P. compressa* L.

Histologic sections of the developing ovules of *P. pratensis* were interpreted by NISHIMURA (11) to indicate the formation of plural embryos from a massive suspensor. "Two or even three buds may develop from the suspensor apparatus." He also "observed where the nucellar cells in the vicinity of the micropyle gave rise to two or three bud-like outgrowths." These were seen in the antipodal region wherein the massive suspensor was also a characteristic feature. Also, nucellar cells lateral to the embryo sac might form embryonic buds. Frequently the growth of the normal embryo sac was inhibited by the growth of the other embryos. These and other similar phenomena were thought to be associated with mechanical injury due to insect attack or to oviposition.

ANDERSEN (2) found that twin embryo sacs arose from sister macrospores in *P. pratensis*. This work was done before the occurrence of apomixis had been suggested as occurring in *Poa* species.

A haploid-diploid relation in *P. palustris* L. (*P. serotina* Ehr.) with 14 and 28 chromosomes, respectively, was reported by KIELLANDER (6), who concluded that in this case the haploid developed from a reduced egg cell. Later he (7) isolated a set of triplets of *P. pratensis*, one member of which had 18 chromosomes. This subhaploid was partially fertile and, after open pollination, gave plants with 15–19 chromosomes.

An evident difference between apomictic strains in their capacities to form several embryos was found by ÅKERBERG (1). Two seedlings from a single seed frequently developed at different times and sometimes were quite diverse in appearance. Later they might appear to be phenotypically alike. Two haploid aberrants ($n = \pm 24$ and ± 29) differed greatly from the normal *P. pratensis* plants.

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, U.S. Department of Agriculture, Soils and Agricultural Engineering, and the Wisconsin Agricultural Experiment Station, Madison.

The frequency of seeds with twin and triplet embryos also varies with the North American strain of Kentucky bluegrass studied (3). Among the different ones observed, ARMSTRONG found that they produced 8, 11, 35, and 42%, respectively, of polyembryonic seeds. In his material he "frequently observed two EMC lying side by side in the prophase stage." These were thought to develop into sister macrospores.

TINNEY (13) found that two embryo sacs may arise independently from two differentiated nucellar cells, but he did not exclude the occurrence of haploids arising from reduced or haploid eggs or the development of triploids as a result of gametic union of an unreduced egg with a haploid male gamete.

ENGELBERT (4) stated that twin embryo "sacs so commonly found in polyploid, polymorphic *Poa* species are, as a rule, developed one from a macrospore, the other from an aposporous cell."

Any of the haploid macrospores of sexual plants of *P. alpina* L. is capable of developing into an embryo sac, with more than one developing occasionally (5). It was concluded that there was no reason to assume that somatic aposporous embryo sacs develop frequently in this species, as often is the case in *P. pratensis*. In the apomictic types of *P. alpina* the embryo sac is formed directly from the macrospore mother cell through mitosis, and, as a result, no reduced macrospores are formed.

Multiple embryo sacs in *P. pratensis* may result in haploid-diploid and in diploid-triploid twins, thus indicating, in one case, the completion of the reduction divisions to form macrospores and, in the other case, the fertilization of the egg of an apomictically formed sac (8).

The frequency of multiple embryo sacs varies from 0 to 63% in ovules of predominantly apomictic, intermediate, and sexual plants of Kentucky bluegrass (9). Apomictic types generally have higher percentages of multiple embryo sacs per floret than do sexual plants.

Material and methods

Fixations were made at 2-day intervals from the emergence of the tip of the panicle until anthesis, after which two additional fixations were made prior to seed ripening. The material selected for each fixation represented the apparently youngest to the most developed florets in several panicles. Later, spikelets of the various sizes and presumed ages were selected from each fixation for histologic study. The usual types of alcohol dehydration and clearing solutions were employed. Sections were cut at 12–25 μ, depending upon the size or age of the florets. Staining was effected with either Haidenhain's iron-alum or Delafield's haematoxylin.

Results

DEVELOPMENT FROM TWO MACROSPORE MOTHER CELLS.—Occasionally two adjacent macrospore mother cells were observed in the regular position in the nucellus (fig. 1). This was especially true of one plant. The reduction divisions occur and two lineal tetrads of macrospores form. Following the regular pattern of events for sexual Kentucky bluegrass plants, two embryo sacs eventually develop (fig. 2). The probable consequence of this type of origin of plural embryos would be two dissimilar plants. Progenies from these would be expected to be highly diverse in plant type.

DEVELOPMENT FROM TWO MEMBERS OF THE EGG APPARATUS.—This relationship perhaps is not widespread in *P. pratensis* owing in part to the frequency of apomictic reproduction in the species.

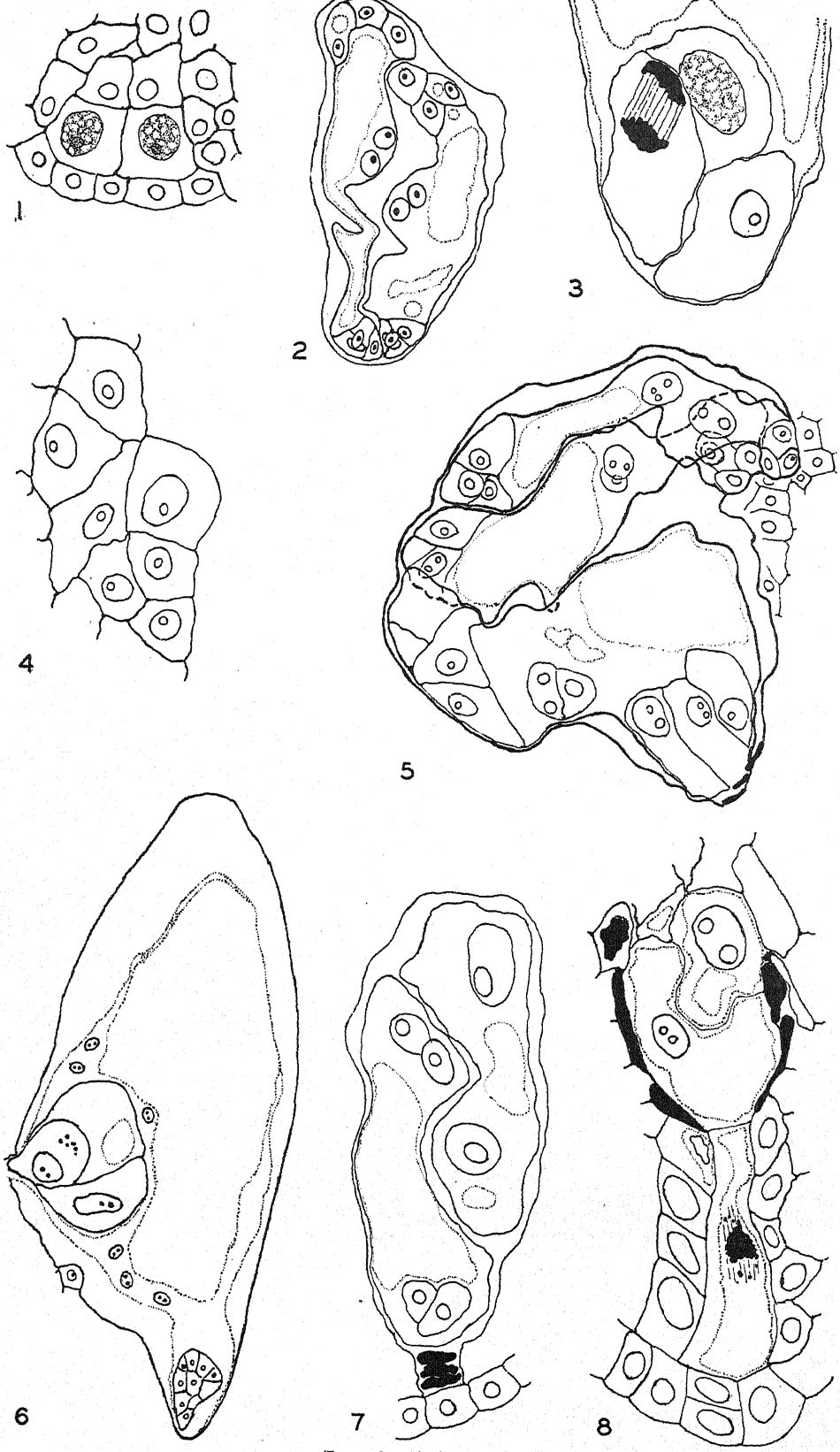
Evidence has been presented elsewhere (9) to show that the reduction divisions take place and that four macrospores result therefrom, the chalazal one eventually developing into the embryo sac. There is evidence that two members of the egg apparatus may sometimes develop, resulting in a haploid-diploid embryo relation.[2] This is in accord with the data given in the companion paper (10) and also with that of earlier workers who have reported haploid-diploid relations in *P. pratensis* twins. There is no evidence that the egg apparatus shown in figure 3 represents this particular combination, since no single embryo sacs were observed with two proembryos or embryos wherein cells with analyzable mitotic-division figures could be found. It appears likely, however, that such was the origin of the haploid-diploid twin set described in some detail elsewhere (10). Although this is true, it remains possible that this twin relation may have arisen from two independent embryo sacs. Each of these would of necessity carry the reduced chromosome complement; one would become diploid through gametic union, whereas the second would develop parthenogenetically because of the stimulative effect of fertilization in the mate.

A second interpretation of figure 3 would indicate the simultaneous development of an egg and a synergid of an embryo sac arising through somatic apospory. If such were the origin, the resulting plants would be expected to be either diploid-diploid or diploid-triploid. The former combination would be the result of the parthenogenetic development of the two cells in question, and the plants should be maternal in appearance and in progeny behavior. The second possible combination, diploid-triploid, would have to involve the fertilization of one member of the egg apparatus and the parthenogenetic development of the other. The resulting set of twins would probably be dissimilar, with the progenies of both members mostly constant.

DEVELOPMENT FROM A MACROSPORE MOTHER CELL AND FROM A SOMATIC CELL OF THE NUCELLUS.—Enlarged, densely cytoplasmic, nucellar cells frequently are observed at the time of the meiotic divisions. Usually, the macrospore mother cell, or its daughter cells, shows definite indications of collapse when such enlarged somatic cells are conspicuous. This is not always the case, however. Instances have been observed where the macrospore mother cells, or the chalazal macrospore, show no indication of necrosis (fig. 7). It is usually in florets wherein the asexual and sexual embryo sacs, or their counterparts, are developing in an essentially synchronous manner that both members appear functional. Twin embryos, one from each embryo sac, would probably develop into phenotypically different mature plants. Obviously, there is great likelihood that they will be different genotypically because of the apparent heterozygosity of the species. They would be either diploid-diploid or diploid-triploid, depending upon whether the egg of the somatic aposporous embryo sac developed parthenogenetically or as the result of gametic union. The progeny from the twin that traces back to the somatic cell, if aposporous reproduction continues, would be expected to be essentially uniform and, if diploid, maternal in appearance. That of the other twin, from the fertilized, reduced embryo-sac egg, would likely differ phenotypically and in breeding behavior from the maternal parent.

It is desirable to indicate another pos-

[2] Relations refer to the total complements, not to *n*-numbers.



(Legend on facing page)

sible combination from this type of multiple embryo-sac development, for the egg cell with the reduced number might develop parthenogenetically as a result of the stimulative effect of the fertilization of the egg nucleus that arose through somatic aposporous processes. Plants produced from embryos that arose in this manner would be expected to be haploid-triploid, a combination that has not been found. Progenies developed from seed of the haploid, if fertile, would perhaps show considerable segregation. The plant from the triploid could be essentially like the maternal parent in breeding behavior but might be quite different phenotypically.

DEVELOPMENT FROM SEVERAL SOMATIC CELLS.—It is not uncommon to observe, in ovules of Kentucky bluegrass, two or more enlarged nucellar cells in close proximity to the macrospore mother cell which is undergoing division (fig. 8). Usually, when such a condition occurs, there are readily recognizable signs of collapse of the macrospore mother cell, as the figure illustrates. In other cases, however, no evidence of necrosis was evident prior to mitosis in the outermost daughter cell. Frequently, if not usually, the division of the innermost daughter cell does not proceed beyond metaphase or early anaphase. No instances were observed that could be definitely interpreted as a macrospore and two or more somatic cells forming functional embryo sacs. Nevertheless, it is possible that such a development might occur.

The phenotypic characteristics of the mature plants developed from multiple embryos from somatic cells would depend largely upon whether or not fertilization of the egg(s) occurred. If such was not the case, the resulting plants would probably be diploid-diploid and similar to the parent. Conversely, if one of the eggs was fertilized and the second developed parthenogenetically, the twins would be triploid-diploid and in all likelihood dissimilar, with the diploid one maternal in appearance.

A third alternative should be recognized. It is possible that both, or even more, of the several egg cells could be fertilized by male gametes carrying diverse factors. If this occurred, it would be logical to expect that the twins, or other multiple embryo combinations, would be dissimilar in morphological characteristics and be triploid-triploid.

SUPERNUMERARY EMBRYO SACS.—The data in the preceding paragraphs pertain to readily classifiable types of plural "macrogametophytes," such as are commonly encountered in *P. pratensis*. In addition to these several distinct classes of multiple embryo sacs of a single ovule, other types of embryo sacs frequently develop. These appear to be secondary. It was not uncommon to observe these secondary embryo sacs, some of which are incomplete and essentially similar to those discussed and figured by HÅKANSSON (5). These structures consist of two or three antipodals and either one or two polar nuclei. Generally, these incomplete embryo sacs taper gradually toward the position generally occupied by the egg apparatus, which is missing in the defective embryo sacs. Here it flares out some-

FIGS. 1–8.—Origin of multiple macrogametophytes in *Poa pratensis:* Fig. 1, two macrospore mother cells. Fig. 2, two embryo sacs. Fig. 3, egg apparatus showing dividing synergid nucleus and egg nucleus at prophase. Fig. 4, enlarged drawing of bud cell shown in figure 6. Fig. 5, three embryo sacs, one proximal at micropyle, others distal. Fig. 6, longitudinal section of ovule showing nucellar bud cell projecting into lumen. Fig. 7, two embryo sacs, four-nucleate one probably developing from macrospore and two-nucleate one from nucellar cell. Fig. 8, collapsing macrospore mother cell with two enlarged nucellar cells.

what and abuts upon a protruding base of several cells. Sometimes, however, these secondary embryo sacs are complete, i.e., containing three antipodals, two polar nuclei, and a regular three-celled egg apparatus.

Although extensive material has been examined in an attempt to complete the gaps in the data at hand, the developmental sequence remains incomplete. It appears desirable, therefore, to record the observations that are fairly definite. Densely cytoplasmic nucellar cells surrounding the gametophytic cavity have been observed in the region adjacent to the confluence of the antipodals and the vascular strands, between the antipodals and the micropyle, and also on the opposite side of the lumen. These regions, together with the antipodals proper, are the loci from which secondary embryo sacs commonly arise. In several ovules, a particularly densely staining cytoplasmic cell with an enlarged nucleus was observed to protrude into the gametophytic cavity (figs. 4, 6). Intervening stages between this and the mature embryo sacs shown in figure 5 have not been observed. Multiple embryo sacs such as those illustrated in the latter figure have been observed commonly. It seems probable that they may explain some of the instances in *P. pratensis* wherein proembryos and embryos are located proximally and even distally to the micropyle in mature seeds.

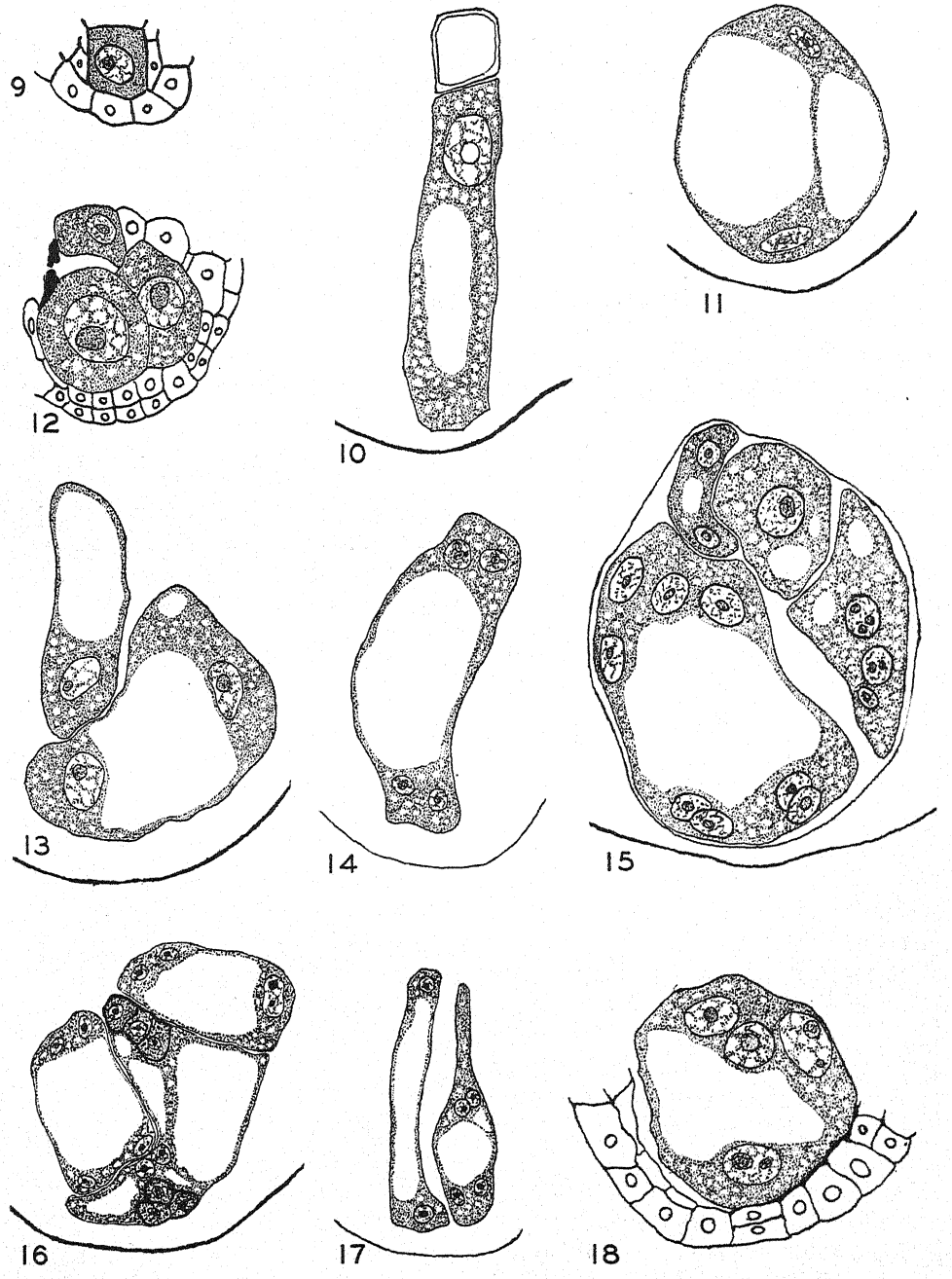
MACROSPOROGENESIS IN THE HAPLOID.—Since earlier studies concerning macrosporogenesis in *P. pratensis* have been limited primarily to biotypes of higher chromosome number, it was considered desirable to examine some of the material of the haploid plant isolated. In none of the limited number of sections available for study were clear nuclear divisions found. Hence it must be assumed from the study of the microsporocytes that the *n*-number equaled 14.

The macrospore mother cell, although smaller in size than those reported elsewhere for diploid plants (9), was similar in shape and in its position under one hypodermal layer of the ovule primordium (fig. 9).

The type of cell with the single large nucleus as shown in figure 10 had not been observed previously. An "empty" cell was located adjacent to the chalazal end of the cell. Usually, the embryo sac is binucleate before a large vacuole of the type shown in this figure appears. The embryo sacs shown in figures 11 and 14 are relatively typical of those occurring in other Kentucky bluegrass ovules containing one embryo sac. Figure 18 is of an atypical embryo sac with a 3 to 1 distribution of the nuclei.

It is uncertain if the dark masses on the left of figure 12 are the remains of the daughter cells of the macrospore mother cell. The three uninucleate cells shown were no doubt derived from the nucellus, since they are mostly located under two tiers of cells of the latter. Figure 13 shows another of the atypical uninucleate-univacuolate cells and a second rather typical binucleate embryo sac. The eight-nucleate embryo sac of figure 15 is rather typical, as is the binucleate one. The uninucleate cell is univacuolate, and the three-nucleate one atypical. The nuclei of the latter differ in size with an accordingly increased number of nucleoli of from one to three. It differs from the other three members of the quadruplet embryo sacs in this respect.

Only the four-nucleate one of the three embryo sacs shown in figure 16 is fairly typical. The upper member contains a giant nucleus. The number and grouping of the cells and nuclei are rather characteristic of Kentucky bluegrass in the



FIGS. 9–18.—Macrosporogenesis in haploid *Poa pratensis* plant: Fig. 9, macrospore mother cell. Fig. 10, elongated, densely cytoplasmic cell in normal position of macrospore mother cell. Fig. 11, two-nucleate developing embryo sac. Fig. 12, three enlarged, densely cytoplasmic, nucellar cells. Fig. 13, two embryo sacs developing, one uninucleate, the other binucleate. Fig. 14, four-nucleate developing embryo sac. Fig. 15, one-, two-, three-, and eight-nucleate developing embryo sacs. Fig. 16, three-nucleate, four-nucleate, and mature embryo sac. Fig. 17, two- and four-nucleate developing embryo sacs. Fig. 18, four-nucleate developing embryo sac.

remaining mature embryo sac. The configuration is, however, quite abnormal.

The two-nucleate macrogametophyte of figure 17 is quite representative of those in other plants of *P. pratensis* examined. The type of the bottle-shaped member on the right had not been seen before.

It is evident that macrosporogenesis can hardly be considered normal for the haploid of the species. Although this is true, some of the developing macrogametophytes examined did not appear to be strikingly different in appearance from those of known fertile apomicts. Misshapen and odd-appearing embryo sacs that are apparently functional occur commonly in the species. The sterility of this plant was perhaps due to genetic factors rather than to the more obvious cytological disturbances.

Discussion

The observations reported in this paper appear baffling when considered without the supplementary data derived from progeny studies from twin and triplet plants of the species (10). They give an impression of indeterminancy. They become less confusing, however, when they are considered in light of the resulting mature plants and their progenies. When so considered, they aid materially in explaining the great morphological variation and chromosomal diversity that prevails in the species. It is this relationship that will be considered in the discussion rather than the more limited variations in macrosporogenesis. These will, however, serve as the basis of discussion for each of the points considered.

When sexual lines of the species are isolated, selected plants from their progenies will, for the most part, continue to yield highly, or even completely, diverse progenies. Exceptions occur wherein constant phenotypic appearance in the subsequent progenies is due to genetic changes in the genome (9). The high incidence of sterility found in many sexual plants of the species affords a mechanism for the elimination of numerous types. When factors or tendencies for apomixis are present, and where sterility would otherwise ensue, the morphological type is maintained through asexual reproduction. Numerous intermediate plants with tendencies toward both types of reproduction also occur. It is with this intermediate class that the data of this paper are concerned primarily.

It is thought from the material studied that apparently viable and plural embryos should develop essentially synchronously. In ovules where two or more embryo sacs commence development at about the same time, it would seem that the zygote most favorably located with respect to nutrient flow would develop at the expense of the other member or members. It is possible also that earlier initiated members may establish an advantage at the expense of other individuals in the same ovule. This interpretation would explain, in part, the existence of some of the numerous undifferentiated proembryos that are observed in mature or nearly mature seeds. Others might be ascribed to the presence of "supernumerary embryo sacs." These proembryos and partially differentiated embryos may not develop to such an extent that they would be noticeable in germinating seeds.

If the above deductions are valid, it follows that embryo sacs, or their initials, that are located somewhat equally with respect to the nutrient supply and develop in an essentially contemporary manner, would constitute the majority of the cases that develop plural embryos evident at germination. It has been shown that both types of embryo sacs—i.e.,

those that arise from macrospores (9) and those that arise from nucellar cells (9, 13, and others)—may be functional. This would offer a reasonable explanation of the marked polymorphism that prevails in the species.

The divergence in gross morphological features of the progeny of a single sexual plant of *P. pratensis* may be considerable. Some workers have suggested that the species is of hybrid origin; this appears quite probable (9). SMITH, NIELSEN, and AHLGREN (12), after studying several thousands of plants, concluded that about 16% of all the plants studied were aberrants. The most plausible explanation of this condition would seem to be the recombination of genetic factors due to sexual processes, the accumulation of factors due to the fertilization of asexually developed eggs by reduced male gametes, and internal chromosomal and/or genetic changes.

Much evidence has been presented during the last fifteen years to indicate and to support the concept of maintenance by apomixis of the morphological types of Kentucky bluegrass established through the indicated processes. The maintenance of the synthesized types occurs in about 84% of the total population of the species (12). This represents the essentially stable apomictic but highly polymorphic complex of biotypes generally evident in the species. New combinations are being repeatedly formed in approximately 16% of the plants of the species. Some of these may become established. They may arise by any one of the processes indicated. Principally the aberrants may arise by the fertilization of eggs having the reduced number of chromosomes or by the fertilization of unreduced eggs produced as a result of somatic apospory. The formation of haploids as a result of the development of an unfertilized member of the egg apparatus into an embryo and the formation of tetraploids through the somatic doubling of the dividing zygote may also occur. Where these are fertile, or at least partially fertile, they also serve as new points from which polyploid series of apomictic and sexual types may arise. In view of the facts concerning progeny behavior from twin and triplet plants, and the presumed modes of origin of macrogametophytes from which these arose, it is to be expected that numerous biotypes, divergent both in morphology and in chromosome number, will occur.

Summary

The origin of multiple macrogametophytes in *Poa pratensis* was studied and the results presented in relation to known breeding behavior of plants from both plural and single embryos.

Eggs developed following meiosis may result in haploid-diploid and diploid-diploid relationships in the plural embryos and in the mature plants. The origin of these plural embryos is (1) from two members of the egg apparatus and (2) from two macrospore mother cells.

Embryo sacs developed from somatic or nucellar cells may form diploid-diploid, diploid-triploid, and diploid-tetraploid combinations of plural embryos and mature plants. The diploid-diploid combinations are considered to arise from two somatic egg cells or from an egg and a synergid of a single embryo sac that develop parthenogenetically. The diploid-triploid combination probably arises in a similar manner; the egg of one embryo sac develops parthenogenetically, whereas that of the second develops as a result of fertilization by a male gamete carrying approximately the reduced chromosome number. Triploid-triploid combinations could form by the

fertilizing of two cells (two eggs or an egg and a synergid). The diploid member of diploid-tetraploid plural embryos might originate in the manner indicated previously for other combinations, the tetraploid by the parthenogenetic development of the egg with a somatic doubling of the chromosomes of the nuclear complement at the time of the zygotic division, or fertilization with an unreduced or restitution male nucleus, or with a high polyploid gamete. The chromosome complement of the male gamete would have to equal that of the egg nucleus. It is considered quite improbable that origin of tetraploids involving gametes would be of common occurrence.

Plural embryos were found to arise from reduced macrospores and nucellar cells in the same ovule. The embryos resulting from these would be diploid-diploid or diploid-triploid, depending upon whether fertilization of only the egg of the reduced macrogametophyte or fertilization of all of the several members, reduced and unreduced, occurred.

"Supernumerary embryo sacs" might develop from nucellar "bud" cells of the wall of the gametophytic cavity. These embryo sacs may be imperfect and nonfunctional, or they may be complete and account for some of the instances wherein proembryos or well-developed embryos are located at various positions other than at or near the micropylar portion of the seed. The observations are incomplete for such a manner of origin of plural embryos.

Macrosporogenesis in the haploid plant was quite similar to that reported for the higher polyploids of the species. The sterility of this plant is undoubtedly due to genetic rather than to evident cytologic disturbances.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ÅKERBERG, E. Apomictic and sexual seed formation in *Poa pratensis*. Hereditas 25:359–370. 1939.
2. ANDERSEN, A. Development of the female gametophyte and caryopsis of *Poa pratensis* and *Poa compressa*. Jour. Agr. Res. 34:1001–1018. 1927.
3. ARMSTRONG, J. M. A cytologic study of the genus *Poa* L. Canad. Jour. Res. (Bot. Sci.) 15: 281–297. 1937.
4. ENGELBERT, V. The development of twin embryo sacs, embryos, and endosperm in *Poa arctica* R. Br. Canad. Jour. Res. (Bot. Sci.) 19:135–144. 1941.
5. HÅKANNSON, A. H. Die Entwicklung des Embryosacks und die Befruchtung bei *Poa alpina*. Hereditas 28:25–61. 1943.
6. KIELLANDER, C. L. Apomixis bei *Poa serotina*. Bot. Not. Pp. 85–95. 1935.
7. ———. A subhaploid *Poa pratensis* L. with 18 chromosomes and its progeny. Svensk. Bot. Tidskr. 36:200–220. 1942.
8. MÜNTZING, A. Further studies on apomixis and sexuality in *Poa*. Hereditas 26:115–190. 1940.
9. NIELSEN, E. L. Cytology and breeding behavior of selected plants of *Poa pratensis*. BOT. GAZ. 106:357–382. 1945.
10. ———. Breeding behavior and chromosome numbers in progenies from twin and triplet plants of *Poa pratensis*. BOT. GAZ. 108:26–40. 1946.
11. NISHIMURA, M. On the germination and the polyembryony of *Poa pratensis* L. Bot. Mag. (Tokyo) 36:47–54. 1922.
12. SMITH, D. C.; NIELSEN, E. L.; and AHLGREN, H. L. A study of ecotypes of *Poa pratensis* L. BOT. GAZ. 108. 1946. (In press.)
13. TINNEY, F. W. Cytology of parthenogenesis in *Poa pratensis*. Jour. Agr. Res. 60:351–360. 1940.
14. ZIMMERMAN, A. Ueber Polyebmryonie bei *Poa pratensis*. Archiv des vereins der Freunde der Naturgeschichte im Mecklenberg. Rostock. 58:107. 1904.
15. ZINN, J. Normal and abnormal germination of grass fruits. Me. Agr. Exp. Sta. Bull. 294. 1904.

ON CARDIOCARPON AND SOME ASSOCIATED PLANT FRAGMENTS FROM IOWA COAL FIELDS

FREDDA D. REED

Introduction

The genus *Cardiocarpon* was established in 1828 by BRONGNIART (3) for some carboniferous seeds which he diagnosed as "fruits comprimés lenticularis, cordiformis ou réniformes terminés par un pointe peu aigüe." Subsequent attempts have been made to break up the genus by employing the term *Cordaicarpon* for seeds known to be related to *Cordaites* and by including in the genus *Samariopsis* those forms in which the wing of the sarcotesta is particularly wide and membranaceous (19). However, since most of the seeds that have been found either are isolated and unattached to vegetative organs and in varying degrees of preservation or are in the form of casts and impressions, there is a consequent lack of constant and definite diagnostic features. Therefore, until more information is available, *Cardiocarpon* remains, and is herein regarded, as a provisional generic term for the reception of carboniferous seeds which are platyspermic with an acute apex and a cordate or sometimes reniform base.

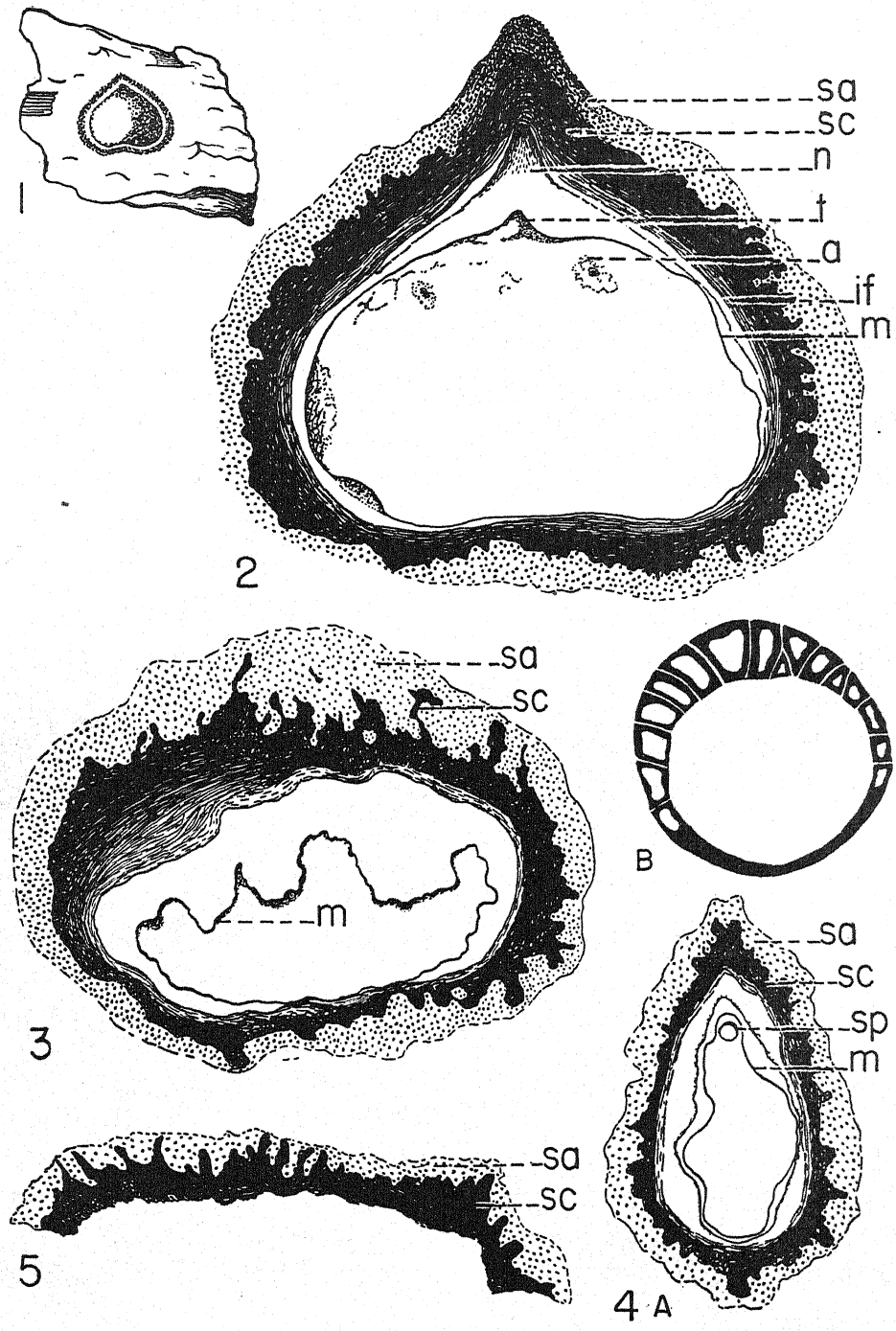
More than seventy species of *Cardiocarpon* have been collected from American coal fields and figured and described by NEWBERRY (14, 15, 16), LESQUEREUX (12, 13), DAWSON (7, 8, 9, 10), ANDREWS (1), WHITE (21, 22), STOPES (20), NOÉ (17), GRAHAM (11), and DARRAH (5, 6). They have been found in geological strata ranging from the Upper Devonian to the Permian, and geographically throughout the range of carboniferous plants in North America. While reinvestigation would doubtless reduce the number of recorded species, there is significance, nevertheless, in their frequency and in their wide geological and geographical range in that both factors indicate for *Cardiocarpon*-bearing plants an important, if not a dominant, place in paleozoic floras.

Material and methods

This investigation is based upon two collections of *Cardiocarpon* from different localities of Iowa. The collections were similar in their mode of preservation in that the seeds were imbedded in calcareous shale. Moreover, the matrix was of such nature that a blow with the rock hammer would shatter it along the cleavage planes into thin flattish pieces. The cleavage planes almost invariably coincide with the flat side of the seed (fig. 1). By so shattering the rocks not only were the seeds revealed but the rock was rendered into pieces of workable sizes without having to section it.

In some instances the seeds were dissected out, coated with cedar oil, which provides a good reflecting surface, and examined for any superficial markings by means of reflected light. The internal structure was determined by grinding the specimens, and at successive levels the surface was polished and the cellular detail was recorded in a series of camera-lucida drawings.

Other specimens were left *in situ*, and the surrounding matrix was ground and polished with the seed. This latter method, although slower, had two dis-



FIGS. 1–5.—*Cardiocarpon*. Fig. 1, impression of *Cardiocarpon* in shale. About natural size. Fig. 2, almost median section of *Cardiocarpon: sa*, sarcotesta; *sc*, sclerotesta; *if*, inner fleshy layer; *n*, nucellus; *t*, tent pole of endosperm; *m*, megaspore membrane; *a*, archegonia. Figs. 3, 4, tangential and transverse sections showing irregular development of sclerotesta. Fig. 4*A*, median longitudinal section showing fern sporangium within megaspore membrane: *sp*, sporangium. Fig. 4*B*, sporangium in detail.

tinct advantages: first, any organically attached structures were preserved and salvaged, and, second, such preparations revealed the structure of other intermingled and associated plant fragments. The latter are interesting of themselves and often furnish clues for the interpretation of the particular specimen that is under investigation.

Investigation

COLLECTION I.—These seeds were collected by HAROLD ALEXANDER, whose account of their origin is as follows: "These specimens are from the rock dump at the mine of the Iowa-Nebraska Coal Co., southwest of Lucas, Iowa. This is located in S.W. $\frac{1}{4}$ of section 23, Jackson township, in the southwestern portion of Lucas County. This mine was closed in 1925 and the workings are now full of water . . . . this mine worked the 'lower coal' which lies within the Cherokee group of the Pennsylvanian system. . . . It is interesting to note that these specimens are from a locality only about twenty miles removed from where the first petrified *Lepidostrobus* was found."

Altogether there were about thirty specimens of *Cardiocarpon* in this collection. Some of them are little more than casts of the endosperm, whereas others, with their integuments more or less intact, are imbedded in calcareous shale and are intermingled with such other plant particles as fragments of wood, leaves, rootlets, sporangia, and spores.

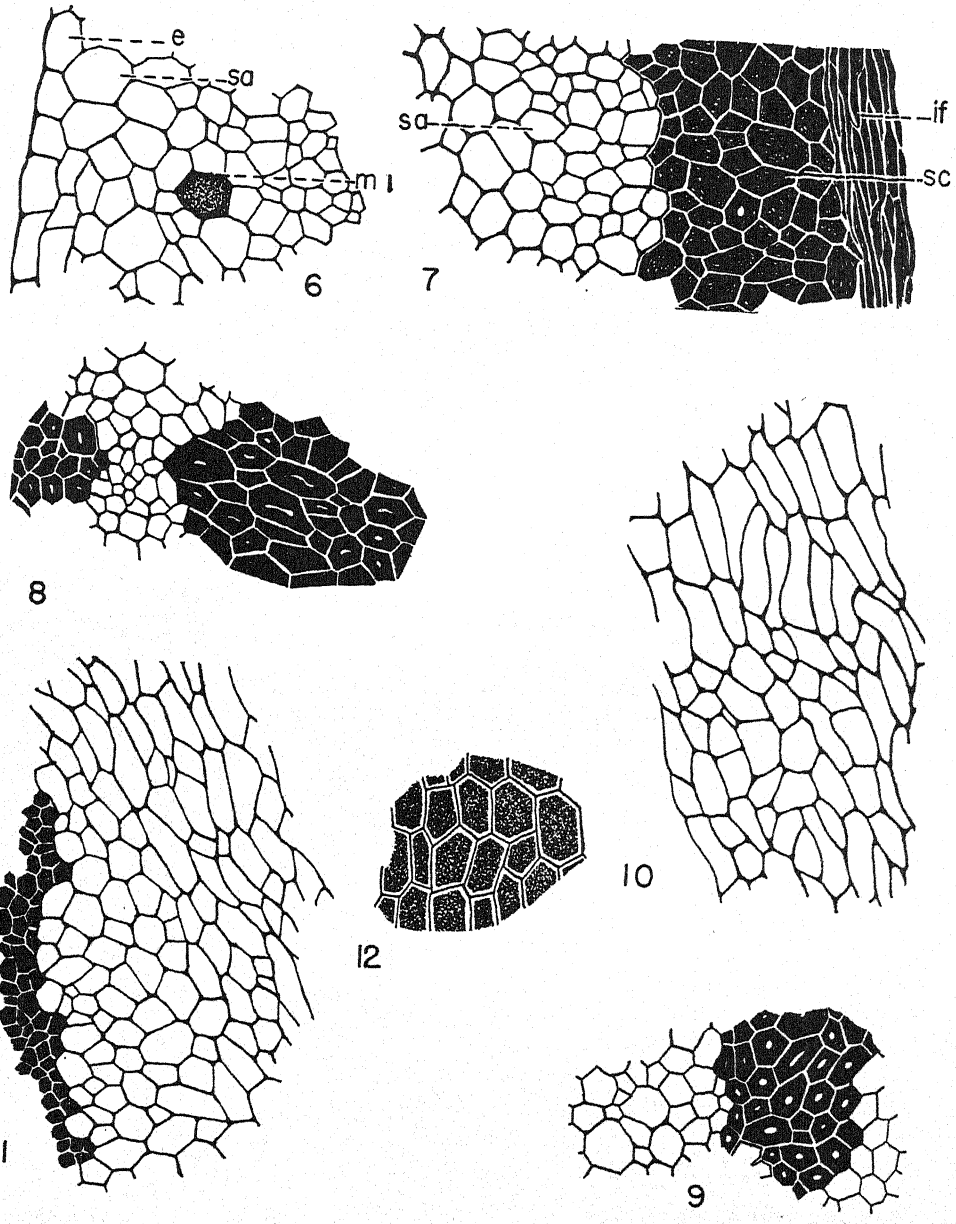
These platyspermic seeds are bilaterally symmetrical. The two planes of symmetry have been designated by Mrs. ARBER (2) as the "primary plane," which is the broad plane, and the "secondary plane," which is at right angles to the broad plane. The specimens show some variation in size; the dimensions, excluding the integument, may range from 7 to 10 mm. broad, from 2.5 to 5 mm. thick, and from 5 to 10 mm. long. The integument as seen in any plane is apparently a uniform layer increasing all dimensions by about 1 mm.

Sections of the seed reveal three distinct layers of the integument: the outer fleshy layer, or the sarcotesta; the stony layer, or sclerotesta; and an inner, less well-defined layer of elongated cells here referred to as the "inner fleshy layer."

The sarcotesta is not preserved in its entirety. For the most part the outer limiting cells have disappeared, leaving no hint as to the actual extent of the layer; occasionally, however, the epidermis, or what appears to be epidermis is encountered (fig. 6). Figures 7, 8, 9, 10, and 11 show details of the sarcotesta at different levels and in different planes. The cells are relatively thin-walled and isodiametric; those abutting the sclerotesta are smaller in diameter, and from this region to the periphery they become increasingly larger. Large cells with dark content, as the one shown at *m* in figure 6, are scattered sparingly and at random throughout the sarcotesta and appear to be mucilage bearing. Toward the micropylar region the cells become thicker walled, progressively elongated, and seem to focalize at the apex (figs. 10, 11).

The sclerotesta consists of exceedingly thick-walled, roughly isodiametric cells (figs. 7, 8, 9, 11). In common with some other described specimens of *Cardiocarpon* (11, 5), the stony layer is irregularly developed, with the irregularities forming spiny projections extending into the sarcotesta (figs. 2, 3, 4*A*, 5).

The inner fleshy layer is the most difficult to interpret, for the limitations of the cell walls are obscure. Moreover, in this zone it is not always possible to distinguish between the cells of the inner fleshy layer and those of the closely ap-



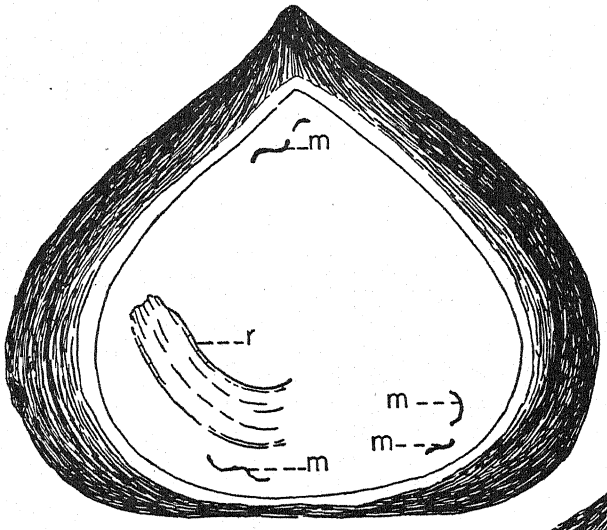
FIGS. 6–12.—*Cardiocarpon*. Fig. 6, portion of sarcotesta: *e*, epidermis; *mi*, mucilage cell. Figs. 7–11, details of integument. Fig. 12, surface view of tapetal cells.

plied nucellus. There is no positive evidence that this is a zone of vascular tissue, yet some of the elongated cells have the appearance of parenchymatous cells of the type that accompany vascular elements. There is also an occasional hint of scalariform markings on some of the walls.

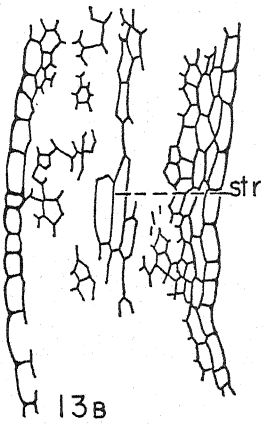
In the genus *Cardiocarpon* the nucellus and integument have been found to diverge near the base of the ovule. In these specimens the course of the nucellus, while it cannot be followed with certainty, does not appear to differ from other members of the group. The tapetal cells form the most conspicuous and characteristic layer of the tissue. Often they are to be seen separated from the rest of the nucellus and in contact with the megaspore membrane. The cells are elongated in the long plane of the ovule and appear to have been filled with some substance that has been preserved as dark-brown material. This material has the same configuration as that of the cells but does not extend to the walls (fig. 12). In these specimens there is no indication of the general structure and nature of the pollen chamber.

As in other paleozoic seeds the megaspore membrane is thick and conspicuous. In a few specimens there has been some shrinkage of the membrane, but in many seeds it appears firm and round and apparently outlines faithfully the shape and extent of the female gametophyte or endosperm. The endosperm as outlined by the megaspore membrane is slightly broader than long and at the micropylar region formed a beak, the "tent pole" of *Ginkgo* and of the Cardiocarpales, extending into the pollen chamber. There are occasional instances of tissue which may be endosperm (fig. 2), but most of the tissue within the membrane is obviously intruded material, as the sporangium in figure 4*A* *sp*, or the rootlet in figures 13*A*, 14, and 15*A*. For the most part the endosperm has been replaced with mineral matter, mostly calcium carbonate, leaving only the faintest remains of the original organic structure.

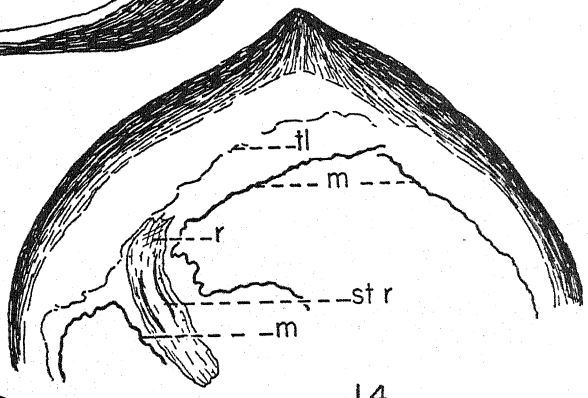
The seed, which has been figured previously (18) and shown here in figure 2, presents an interesting study. The megaspore membrane of this specimen seems intact, and there is no indication of invaded foreign material. The region of the endosperm is almost completely mineralized, with indistinct light-brown markings representing the organic material. Just below and at either side of the tent pole are two more or less defined areas bounded by delicate brown lines and each containing a somewhat dense, centrally located body. The position and size of these areas strongly suggest the central cells of archegonia with the dense bodies as their nuclei. While the markings which represent the organic structure of the endosperm appear to be determined by crystallization rather than by the position of the original cell walls, yet it is possible that the process of crystallization may have been influenced by the density of the protoplasm in which the mineral was being precipitated. Since, by analogy with extant gymnosperms, there would be a decided variation in the density of the central cell and the surrounding cells of the endosperm, it seems highly probable that these bodies replace and, therefore, record the position of the archegonia. Furthermore, that the presence of these areas was not wholly fortuitous is attested by the fact that the same topography was observed in the endosperm of more than one ovule. As was suggested in the former article (18), if one assumes a parallel development of the endosperm of *Cardiocarpon* with that of *Ginkgo*, then the two



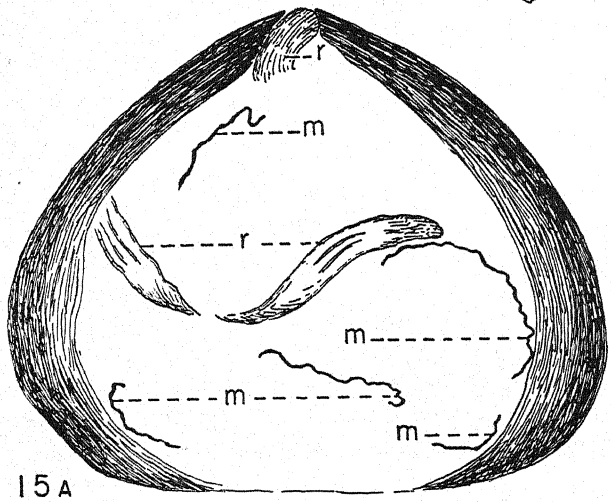
13A



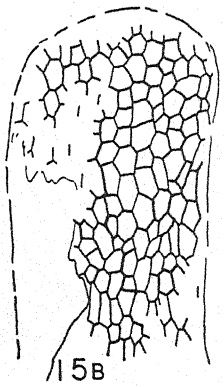
13B



14



15A



15B

FIGS. 13–15.—*Cardiocarpon*, series of diagrams showing position of rootlet within region of endosperm: *r*, rootlet; *st r*, stele of rootlet. Figs. 13*B* and 15*B* are details of rootlet from Figs. 13*A* and 15*A*.

dense bodies would occupy the position of the nuclei of the fertilized eggs. Were they the egg nuclei, that is, before fertilization, one would expect to find them at the micropylar end of the central cell rather than centrally located as these appear to be.

Another interesting specimen is the one shown in figures 13*A*, 14, and 15*A*. During the process of grinding down this seed there was observed within the megaspore membrane some plant tissue, as shown in figures 13*B* and 15*B*. The shape and position of this tissue, together with the general aspect of the cells composing it, suggested that it might be embryonic and that it might be either a tangential section through a cotyledon or perhaps through the hypocotyl. From this stage on the grinding proceeded slowly, and the median plane was approached cautiously. Observations and drawings were made at intervals of 10–20μ. When the median plane was reached, the seed was turned over and ground from the opposite side until the "tissue" was visible; then the specimen was mounted.

The two faces of the finished preparation (figs. 13*A*, 15*A*) conclusively demonstrate that the "tissue" is not an embryo or pieces thereof but is an intruded rootlet. Also these two faces not only reveal the nature of the rootlet but indicate its course through the endosperm—a course that is corroborated by the serial drawings made during the progress of the preparation. In figure 15*A* a tangential portion of the rootlet is shown in the region of the nucellus where it had apparently entered from the micropyle; figure 15*B* is a detail of this portion. From this region the rootlet appears to have pursued a downward but winding course. In the plane illustrated in figure 14 there is a rupture in the megaspore membrane, and the rootlet is seen partly within the confines of the endosperm. Again, in figure 13 the portion at *r* is wholly within the endosperm, and its winding or bending course is further indicated. The tortuous position of the rootlet provides both tangential and radial views of it in one plane of the seed. The cellular preservation is so incomplete, however, that there is only a suggestion of its anatomy.

Despite the lack of conclusive evidence there are certain observable facts with regard to this tissue: (*a*) it is one continuous organ from the region of the micropyle through the nucellus and megaspore membrane, into and winding through the endosperm; (*b*) throughout its length a uniform diameter is maintained; (*c*) only primary tissues are apparent, with little evidence of maturation of cells; and (*d*) the incipient vascular elements are apparently central. A summation of these facts seems to establish the identity of this tissue as being that of an intruded rootlet.

If further evidence were needed, it seems amply supplied by the masses of apparently inextricable rootlets of comparable dimensions and anatomy which are sometimes observed in the matrix exterior to the seeds and by the fact that there are other recognizable intruded plant fragments, as the sporangium shown in diagram in figure 4*A* and in detail in figure 4*B*.

COLLECTION II.—The pieces of calcareous shale of this collection were kindly and generously supplied by L. R. WILSON of Coe College. He writes of their source: "The material was collected from the strip mine of the What Cheer Clay Products Company. The Iowa Geological Survey does not seem to have studied the section very thoroughly in the vicinity of What Cheer, Keokuk County. However, its position in the Des Moines Series appears to be rather low."

Of this material, there were dozens of flattish pieces of limestone, each one studded with two or more seeds. In one piece, which was about 3 inches square by $\frac{1}{3}$–$\frac{1}{4}$ inch thick, portions of fifteen different seeds were exposed, and, upon grinding the piece of rock still others were revealed. Intermingled with *Cardiocarpon* were layers of leaves and some stem apices, both of which appear to be of Cordaitean origin, as well as numerous other pieces of roots, stem, sporangia, and spores.

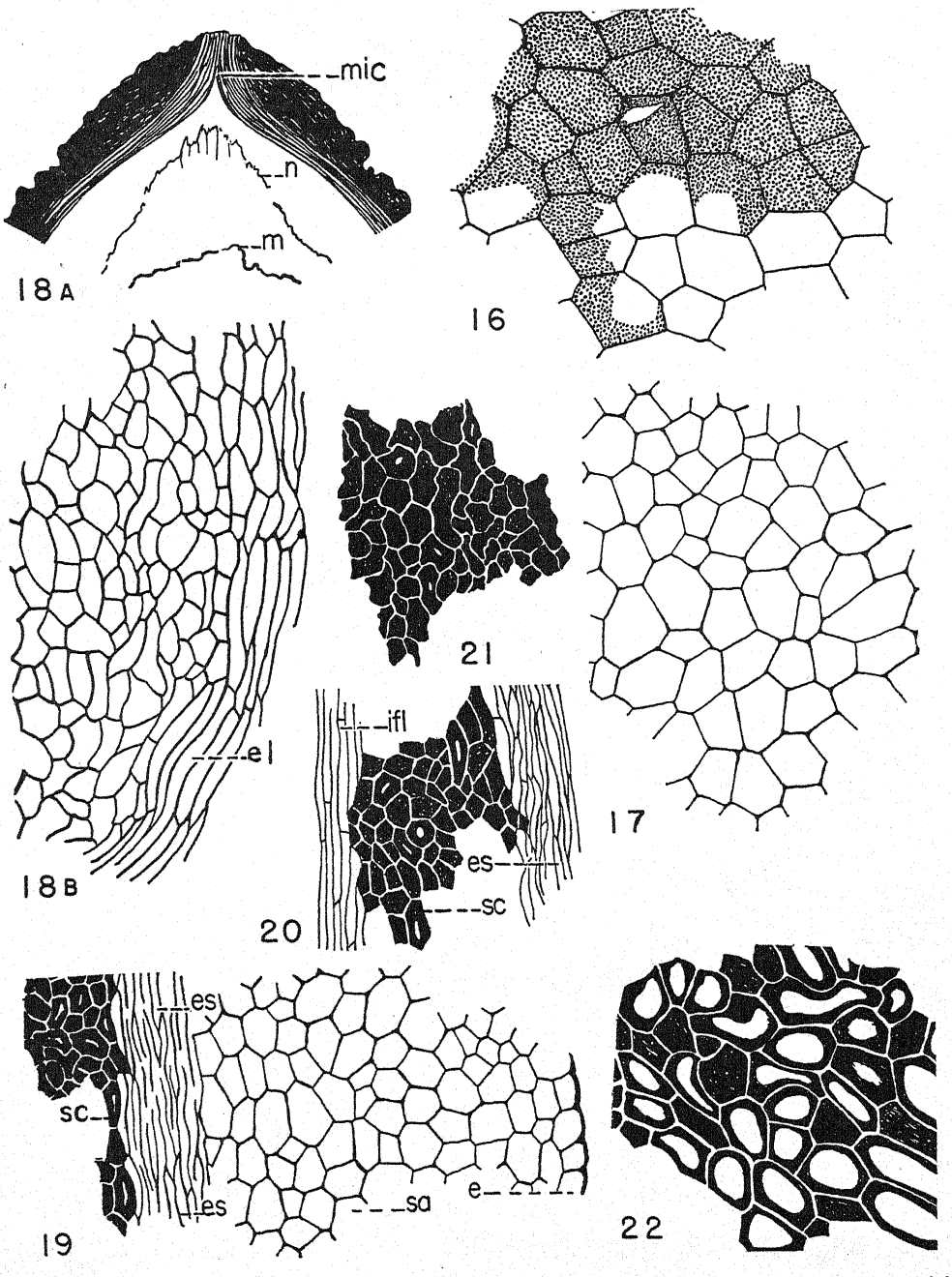
More than a hundred seeds have been examined and have been observed in almost as many planes. In their size, their gross topography, and their internal anatomy, in so far as it could be determined, these specimens bear a striking resemblance to those of the former collection. Only those seeds or such portions of them possessing certain features that make a contribution to our knowledge of the group will be described.

Many specimens were found with the integument apparently intact, and from them it was possible to get good views of the sarcotesta and the characteristics of the several layers of cells composing it. In a surface view the epidermal cells appear golden brown, but in section the material responsible for it is clearly shown to be limited to the outer or superficial walls of the cells, and, therefore, it is thought to represent the cutin (fig. 16). With one exception the remaining layers of the sarcotesta, both in extent and in size of the cells, are similar to the comparable layers of the seeds described above. The exception is in the elongated cells adjacent to the sclerotesta (figs. 19, 20). While no markings were observed on the walls of any of these elongated cells, it is thought that they indicate the presence and position of vascular elements in the outer fleshy layer, as shown in the reconstruction (fig. 26). Moreover, these elongated cells are apparently confined to narrow zones or discrete strands, for in most places the cells of the innermost layers of the sarcotesta are not elongated but are isodiametric (figs. 7, 9).

The sclerotesta (figs. 19, 20, 21, 22), in the size of the cells composing it, in its extent, and in its configuration, is in general aspect conformable with that layer of the former specimens; still, some additional features are revealed because of the more complete preservation. For most of the extent of the sclerotesta the cells are isodiametric, but about the chalazal region and again in the vicinity of the micropyle they are irregular, somewhat elongated, and interlocking. Nor are the cell walls always as thick as is shown in figures 19 and 20, in which the lumen, in section, appears to be reduced to a narrow slit or is almost nonexistent. Occasionally an ovule is encountered in which, throughout the region of the sclerotesta, the walls are much less thick and the lumen is a generous proportion of the cell (fig. 22).

Elongated cells characterize the inner fleshy layer, as was described above (fig. 20). This tissue is demonstrated to be more extensive than was evident in the former specimens, for it not only completely surrounds the nucellus but extends into and lines the micropylar canal (figs. 18*A*, 18*B*).

In a number of instances there is very good evidence of the divergence of the integument and the nucellus, and in each case the pattern conforms to that shown in the reconstruction (fig. 26). Not only is there very little preservation of the nucellar tissue, but the few remaining cells show considerable disintegration, as if the layer had broken down before petrifaction occurred; there is evidence of a



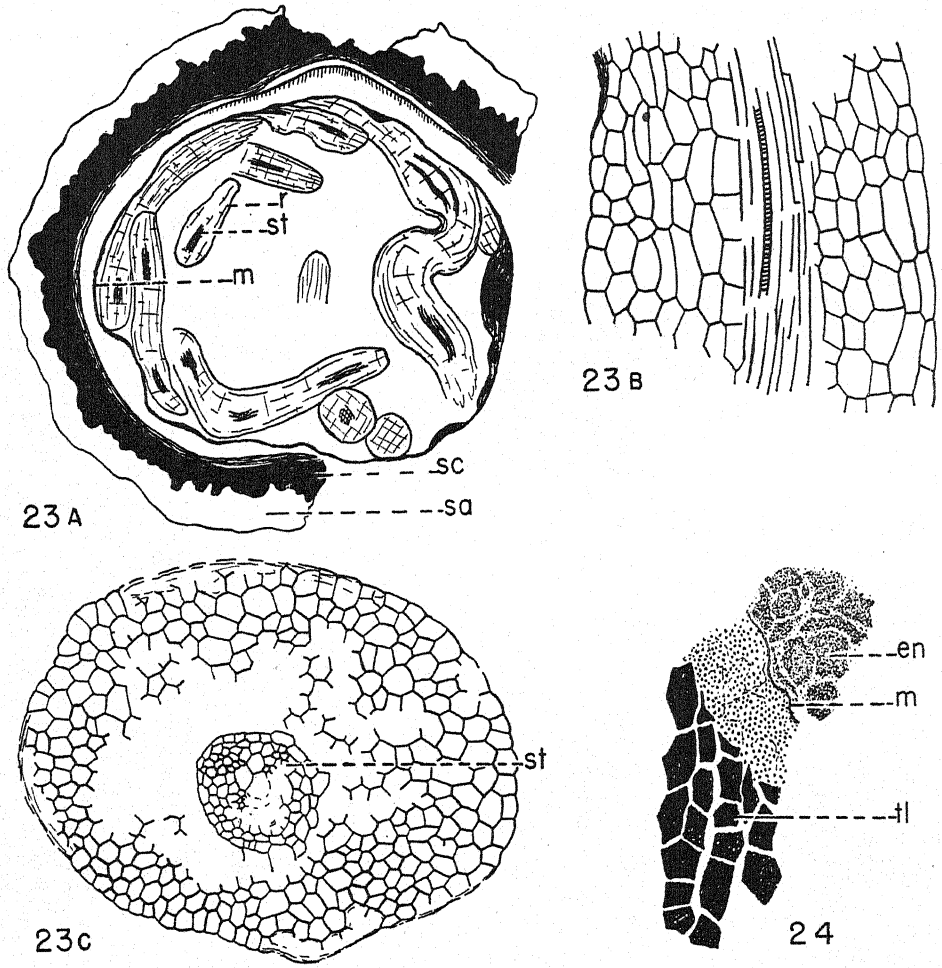
FIGS. 16–22.—*Cardiocarpon*. Fig. 16, surface view of epidermal cells of integument. Fig. 17, tangential section through sarcotesta. Fig. 18*A*, diagram of micropylar region of integument. Fig 18*B*, detail of inner fleshy layer from *A*: *mic*, micropyle; *el*, elongated cells of integument which line micropylar canal. Figs. 19, 20, sections through integument: *es*, elongated cells of sarcotesta; *ifl*, inner fleshy layer. Fig. 22, portion of sclerotesta from seed in which walls of sclerotesta were not so thick.

prominent nucellar beak (figs. 18*A*, 26). The tapetal cells of the nucellus verify the description recorded for that layer from the seeds of collection I.

Almost every specimen examined, whatever the degree of preservation, has some portion of the pronounced megaspore membrane (figs. 23*A*, 25, 26); and in many of them it appears to be intact. Within the megaspore membrane there is no tissue that is manifestly megagametophyte, nor is there any tissue that can be demonstrated as being embryo.

In some instances there is within the megaspore membrane some organic material which is conceivably endosperm. There is some indication of cellular organization of this material, but it is without cell walls and is of such an amorphous nature that no clue is left as to its original structure (fig. 24).

It is both interesting and curious that



FIGS. 23–24.—*Cardiocarpon*. Fig. 23*A*, rootlet in region of endosperm. Fig. 23*B*, detail of rootlet in longitudinal section. Fig. 23*C*, rootlet in transverse section. Fig. 24, portion from tangential section showing indication of cellular organization of endosperm: *en*, endosperm.

in fully half these seeds there were lengths of rootlets which, in their proportions and in their structure, in so far as they are demonstrable, are comparable to the one shown in figures 13, 14, and 15. In some specimens only a few short lengths were visible, whereas in others long pieces of rootlets apparently wound around and back and forth throughout the region of the endosperm (fig. 23*A*). Again, some rootlets are clearly within the megaspore membrane (fig. 23*A*), while others are found inserted between the integument and nucellus. Moreover, in some cases the rootlets seemed to have entered from the micropyle; in others pieces of rootlets are associated with ruptured places in the integument near the chalazal end of the ovule, as if they had made their entrance or exit from that region. Regardless of its position within the ovule or of its fragmentary condition, the rootlet maintains a constant diameter and apparently uniform structure (figs. 23*B*, 23*C*), thereby removing any possibility of its being regarded as an embryo or portion thereof.

Some associated fragments

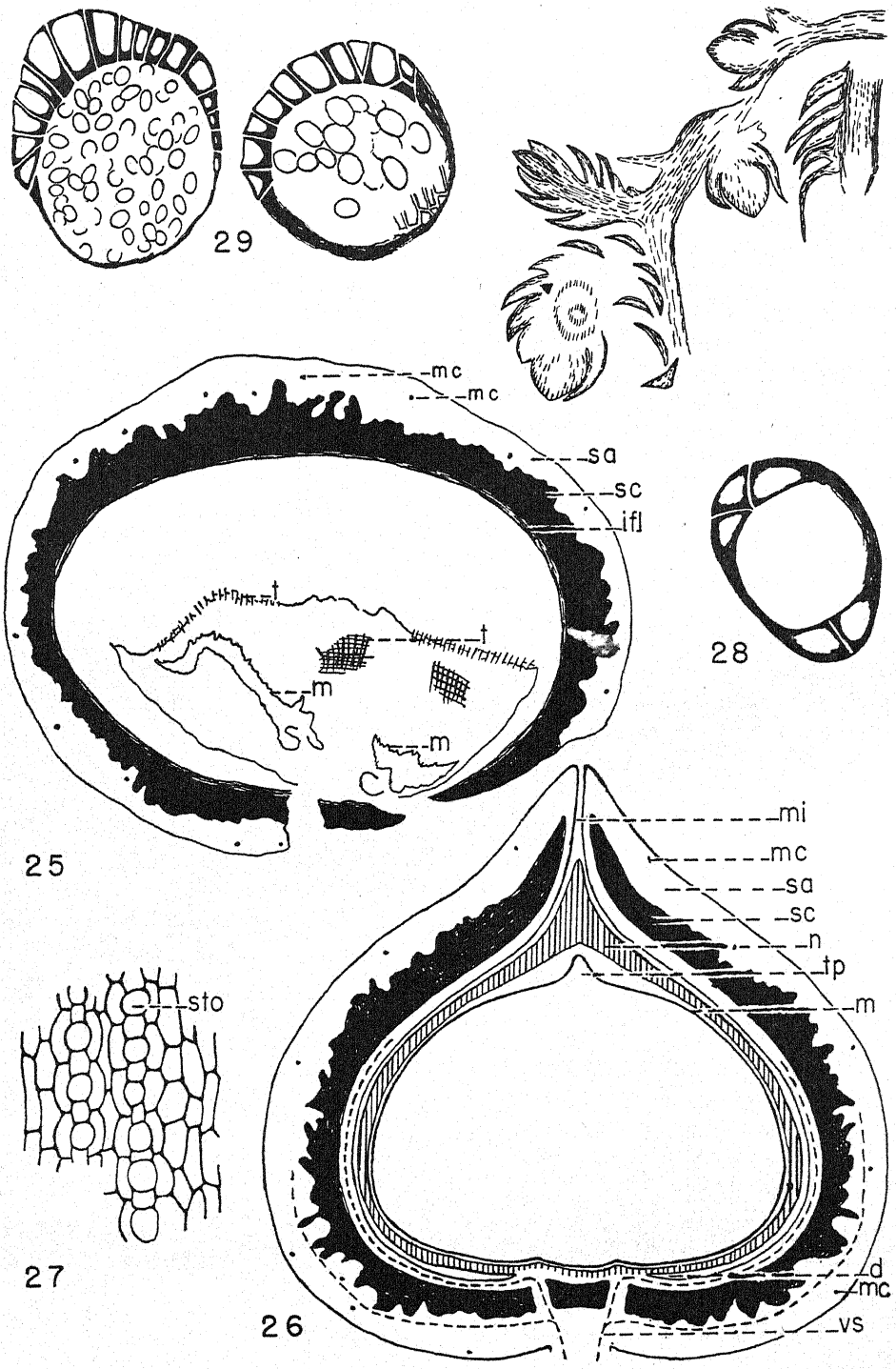
Cordaitean leaves.—Of the associated plant fragments, the most conspicuous and the most abundant were some leaves of *Cordaites*. These were found stratified throughout the pieces of limestone and were arranged layer upon layer. Their size, parallel venation, and the longitudinal strands of sclerotic cells establish these leaves in the genus *Cordaites*, but beyond that the preservation is too meager to admit specific identification. Nevertheless, certain features are worthy of record because of their value in subsequent investigations and also because these leaves may prove to be a link in establishing the identity and structure of the vegetative organs that produced the above-described specimens of *Cardiocarpon*.

The leaves were relatively large. Some of the pieces had a constant width of about 1 inch for 2 or 3 inches in length, and they must then have been much longer. Preservation was limited to the sclerotic and cuticularized cells, and it is the latter that provides the pattern of the epidermis (fig. 27). Areas like the one shown in figure 27 are extremely limited, but they do indicate two characteristics of the epidermis: (*a*) there is a definite and regular arrangement of the stomata and (*b*) in surface view the guard cells are not visible; therefore, the stomata must have been sunken.

Stem apex.—A few stem apices were encountered, one of which is shown in figure 25. No stem tip was found in organic connection with the Cordaitean leaves or with the specimens of *Cardiocarpon*, yet all three—the stem tip, the mature leaves, and the ovules—have certain cell characteristics in common, and it is believed that all may have been part of one plant. As in the case of the leaves there is so little preservation that only the general topography of these stem tips is evident. The closely imbricated arrangement of the small leaves or bracts indicates that these tips were buds. Some buds appear to be strictly vegetative, while interspersed among the appendages or bracts of other tips were microsporangia filled with innumerable microspores.

Discussion

These specimens of *Cardiocarpon* seem to be conformable, in so far as they are preserved, with *Cardiocarpus spinatus* Graham, a species based upon two specimens from Richland County, Illinois (11). Darrah (5) has described some similar seeds from the vicinity of Wau-



FIGS. 25–29.—Figs. 25, 26, *Cardiocarpon*. Fig. 25, proximity and relative size and proportions of *Cardiocarpon* and vegetative tip of *Cordaites*. Fig. 26, reconstruction of median longitudinal section. Fig. 27, portion of epidermis of *Cordaites* leaf: *sto*, stoma. Figs. 28, 29, some associated fern sporangia.

kee, Iowa; and, while he employs the generic term *Cordaicarpus* instead of *Cardiocarpus*, he believes his specimens to belong to the same genus and species as those described by GRAHAM. However, inasmuch as GRAHAM's specimens were incomplete and his specific term was derived from the spiny character of the stony layer of the integument, a character which would not be visible if the seed were entire, it seems questionable whether the term *spinatus* is appropriate. Furthermore, both NEWBERRY (14, 15, 16) and LESQUEREUX (12, 13) have figured specimens of *Cardiocarpon* which, from the illustrations, are so much like the recently described ones that a comparative study would doubtless provide an already established specific name. The descriptions of the earlier investigators did not include cell structure and anatomy but were based upon superficial characters of size, shape, and markings. By a comparison of such superficial characters, the above-described specimens appear strikingly like *Cardiocarpus affinis* Lesq. Therefore, except for retaining BRONGNIART'S (3, 4) generic term *Cardiocarpon*, the same name, *Cardiocarpon affinis* Lesq., is here provisionally applied.

Summary

1. The plant fragments herein described were found in two collections of shaly limestone. Both collections were from the Upper Carboniferous: collection I came from a coal mine near Lucas, Iowa; collection II, from a strip mine in the vicinity of What Cheer, Iowa.

2. Of the plant fragments in both collections, *Cardiocarpon* was the most conspicuous. The description and reconstruction of *Cardiocarpon* are based upon an investigation of more than a hundred different specimens.

3. No specimen of *Cardiocarpon* was found attached to vegetative organs. They are cordate, platyspermic seeds and measure about 1 cm. wide, by 1 cm. long, by 5 mm. thick. There is a single integument of three layers: sarcotesta, sclerotesta, and inner fleshy layer. There is evidence of a double vascular system, one series in the sarcotesta and one in the inner fleshy layer.

4. Some specimens appear to have archegonia. Within the endosperm of many of them there is extraneous and intruded plant tissue.

5. These specimens are apparently conformable with *Cardiocarpon affinis* Lesq., and, as such, have been provisionally designated.

6. The associated fragments are: leaves of *Cordaites;* stem tips with buds, in some of which are found microsporangia, of *Cordaites;* some unidentified rootlets; and some unidentified fern sporangia.

MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MASSACHUSETTS

LITERATURE CITED

1. ANDREWS, E. W. Description of fossil plants from the Coal Measures of Ohio. Rept. Geol. Surv. Ohio, no. 2. Paleontology 2:413–426. 1875.
2. ARBER, AGNES. On the structure of the paleozoic seed *Mitrospermum compressum* Will. Ann. Bot. 24:491–509. 1910.
3. BRONGNIART, ADOLPH. Prodrome d'une histoire des végétaux fossiles. Paris. 1828.
4. ———. Recherches sur les graines fossiles silicifiées. Paris. 1881.
5. DARRAH, W. C. The fossil flora of Iowa coal balls. III. *Cordainthus*. Bot. Mus. Leaf. Harv. Univ. 8:1–20. 1940.
6. ———. Studies of American coal balls. Amer. Jour. Sci. 239:33–53. 1941.
7. DAWSON, J. W. On the flora of the Devonian

period in northeastern America. Quart. Jour. Geol. Soc. 18:296–330. 1862.
8. ———. Further observations on the Devonian plants of Maine, Gaspé, and New York. Quart. Jour. Geol. Soc. 10:458–470. 1863.
9. ———. Synopsis of the flora of the Carboniferous period in Nova Scotia. Canadian Nat. and Geol. 6:451–457. 1865.
10. ———. Geological history of plants. New York. 1896.
11. GRAHAM, ROY. Pennsylvania flora of Illinois as revealed in coal balls. II. BOT. GAZ. 97:156–168. 1935.
12. LESQUEREUX, LEO. New species of fossil plants, from the anthracite and bituminous coal-fields of Pennsylvania. Boston Jour. Nat. Hist. 6:409–431. 1854.
13. ———. Description of the coal flora of the Carboniferous formation in Pennsylvania and throughout the United States. Second Geol. Surv. Penn. 3. 1884.
14. NEWBERRY, J. S. Fossil plants from the Ohio coal basin. Ann. of Sci. 1:106–108. 1853.
15. ———. New species of fossil plants from Ohio. Ann. of Sci. 13:152–153. 1853.
16. ———. Description of fossil plants. Rept. Geol. Surv. of Ohio. 1:359–385. 1873.
17. NOÉ, A. C. Pennsylvania flora of northern Illinois. Bull. 52. Ill. State. Geol. Surv. 1925.
18. REED, FREDDA D. Structure of some Carboniferous seeds from American coal fields. BOT. GAZ. 100:769–787. 1939.
19. SEWARD, A. C. Fossil plants III. Cambridge Univ. Press. 1917.
20. STOPES, MARIE C. The "Fern Ledges" Carbonifero s flora of St. John, New Brunswick. Dept. Mines, Geol. Surv. Canada Mem. 41. 1914.
21. WHITE, DAVID. Fossil flora of the Lower Coal Measures of Missouri. U.S. Geol. Surv. Monographs. Washington. 1899.
22. ———. The stratigraphic succession of the fossil flora of the Pottsville formation in the southern anthracite coal fields. Penn. 20th Ann. Rep. U.S. Geol. Surv. for 1898–99. Washington. 1900.

CONTROL OF FLOWER FORMATION AND FRUIT SIZE IN THE PINEAPPLE

J. VAN OVERBEEK[1]

Introduction

Auxins affect, and under certain conditions control, a variety of physiological processes. Among these may be mentioned elongation of stems, formation and growth of roots, development of lateral buds, abscission, water uptake, protoplasmic streaming, and enzymatic processes. In general, one may say that auxins exert their effects on higher plants as a whole rather than on a specific group or species. An exception seems to be flower induction in the pineapple. It has been shown, so far, that only this plant and perhaps some other bromeliads[2] respond to these substances by forming flowers. The possibility exists, however, that auxins have a general role in flower formation, even though this has not been demonstrated as yet. It was therefore felt that a study of flowering in the pineapple might give rise to information applicable to the control of flowering in general.

The fact that auxins do not induce flowering when applied to most plants could be interpreted as a lack of relationship between auxin and flowering. But it has been shown (11) that applications of auxins to cuttings are without effect unless they can act co-ordinately with substances produced by the leaves. These substances were later identified as certain sugars and nitrogenous compounds (13). In other words, the part auxin plays in floral initiation may be

[1] The assistance of HÉCTOR J. CRUZADO, ELBA SANTIAGO DE VÁZQUEZ, and GUILLERMO DÁVILA is gratefully acknowledged.

[2] *Billbergia* and some other bromeliads respond to ethylene (4), as do pineapples (8, 3).

masked by the absence of other requisite factors.

FLOWER FORMATION IN THE PINEAPPLE PLANT.—During its vegetative phase of growth, the axis of the pineapple plant remains short, and the apex continues to produce foliage leaves. Without treatments this vegetative apex may change, during the winter months, into one which produces an inflorescence. A description of this process has been given by COOPER (3). When flower formation takes place, the formation of foliage leaves is halted and the axis begins to elongate (fig. 1).

One may distinguish between pineapple varieties of which a large percentage of the plants flower at normal flowering time and others which are characterized by a low percentage of flowering. Among the first the Red Spanish, the principal commercial variety in Puerto Rico, may be named. During the winter season not only all mature plants but also many immature plants flower. The flowering of immature plants is highly undesirable.

The Cabezona is a variety of the opposite type. During the flowering season only a relatively small percentage of the mature plants flower, and rarely is a flower formed on an immature plant. In our experience the Cabezona flowers very infrequently during the first year after planting, about 25% of the plants produce flowers during the second year, and the remaining plants, although of mature size and capable of bearing large fruits, will produce in later seasons. It is not uncommon to find plants which produced their first fruit during their fifth season. This natural tendency to limited flowering makes the Cabezona highly suitable for experiments with flower-inducing substances. Delayed flowering is a liability from the commercial standpoint at present, but this characteristic can be turned into an asset, as will be shown in the discussion.

Methods

The Cabezona, which is the commercial variety of the Lajas Valley, was used for the experiments here reported. Preliminary experiments were carried out at Mayagüez, but the bulk of the work was done at the Ortiz[3] farm at Lajas. There the average rainfall is 29 inches annually, with a dry season occurring during the winter and spring months (5). The plants were 14 months old when the work was begun and 28 months old when the present experiments were terminated.

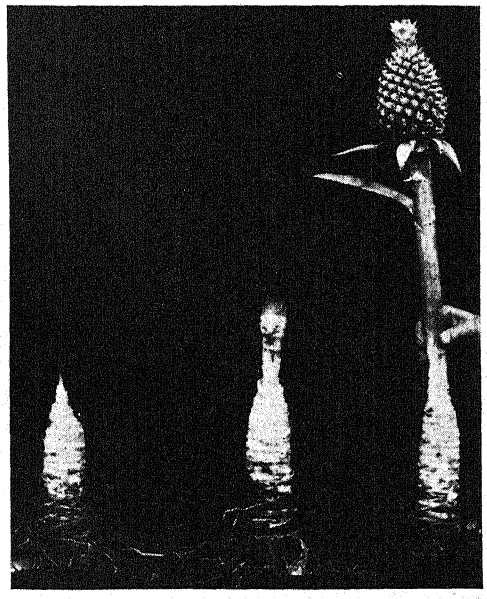


FIG. 1.—Different stages of elongation of axis of Cabezona pineapple. Plants 27 months old. Leaves stripped. *Left*, vegetative stage. *Center*, early (natural) flowering stage, about 7 weeks after flower initiation. *Right*, 3 months and 20 days after treatment with 10 p.p.m. naphthaleneacetic acid (NA). Photo, December 26.

[3] The author is indebted to Dr. A. ORTIZ ROMEU, who generously made part of his best plants available for experimentation.

Applications of flower-inducing substances were made to the center of the plant. Each plant was treated only once. In the early experiments a pressure spray pump was used, and leaves around the center were also sprayed. In later experiments the solutions were poured in the center of the plant with a small beaker. In both instances 50 cc. were applied per plant. The use of Carbowax added to the solutions was found to be of no benefit. Nontreated controls alternated with treated plants in every row.

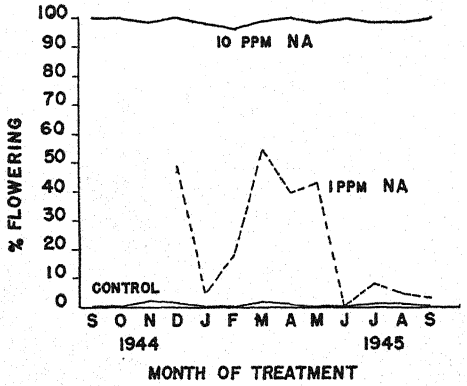


FIG. 2.—Flower induction in Cabezona pineapple throughout year by single treatment with naphthaleneacetic acid (NA). Each plant received 50 cc. of solutions. Plants were 2 years old (after planting) in September, 1945, when last treatment was made. Each point represents average of 40–50 plants.

Naphthaleneacetic acid (NA) was the principal compound used. An aqueous stock solution of 0.1% was carried to the field, where further dilutions were made with distilled water. The acid of this stock solution was neutralized with KOH, NaOH, or NH_4OH in order to prevent the NA from crystallizing. No difference in flower-inducing ability was found between the K, Na, or NH_4 salts of NA, or between the salt and the acid. Another series of experiments was performed using 2,4-dichlorophenoxyacetic acid (2,4-D), neutralized as indicated above, as a flower-inducing agent.

Observations were made once per month, and counts for flowering were made 2 months after treatment. All fruits mentioned were first fruits and not ratoons. When statistical analyses were made, the methods outlined by SNEDECOR (9) were followed.



FIG. 3.—Flower formation in Cabezona pineapple 7 weeks after treatment with 10 p.p.m. NA. At time of treatment plant was 24 months old. Photo, November 12.

Results

RESPONSE TO FLOWER-INDUCING AGENTS THROUGHOUT THE YEAR

When the investigations were begun, no data on flower induction by means of chemical treatments throughout the different seasons were available. From Hawaii a single case was reported in which flowering 4 months ahead of the normal time was obtained (1), and from Florida it was reported that NA treatments given during July, August, and September were not successful, while

those given during October were (2). As has been shown in a preliminary note (10) and in figure 2, NA will induce flowers in every month of the year. Throughout the year the plants responded with great regularity to 10 p.p.m. NA, which caused practically 100% flowering. Flowers became externally visible approximately 7 weeks after treatment (fig. 3), and fruits were ready for harvesting approximately 5 months after treatment (fig. 4).

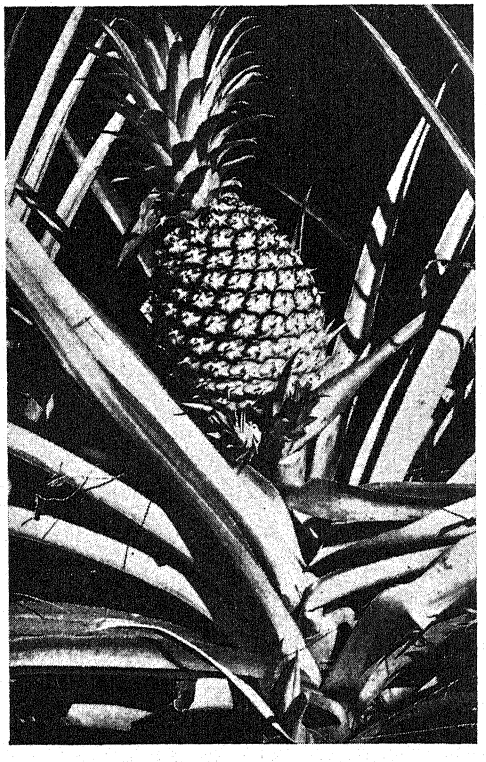


FIG. 4.—Fruit of Cabezona pineapple at harvest (December 19; out of season) 5 months after treatment (July 21). At harvest, plant was 27 months old, had 40 mature leaves, and fruit weight of 4.7 kg. This plant, and consequently its fruit, is larger than average for its age (see fig. 8 and table 2). Note "slips" below fruit, which are used for propagation.

A concentration of 1 p.p.m. appears to lie just at the threshold of flower induction (fig. 2), while 5 p.p.m. appear to be just as effective as 10 p.p.m. (fig. 5). The greater response to 1 p.p.m. during the period from December to May (fig. 2) is most likely attributable to a period of relative drought and not to changes in sensitivity. During dry weather there is less water in the center of the plant than during periods with frequent rainfall, and hence the applications will be diluted to a lesser degree and there will be less overflow during the dry season.

When applied in quantities of 50 cc., the amount of growth regulator used is,

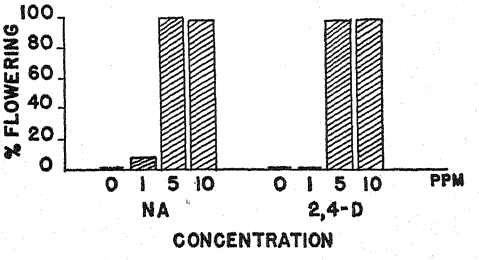


FIG. 5.—Flower induction as function of concentration of NA and 2,4-D, applied by a single treatment in July, when plants were 22 months old. Each value represents average of 40–50 plants.

for 1, 5, and 10 p.p.m., respectively, 0.05, 0.25, and 0.5 mg. per plant. Within certain limits it is immaterial whether NA is applied in relatively strong or weak solutions as long as the total amount applied remains the same (table 1).

RELATION BETWEEN NUMBER OF LEAVES AND FRUIT WEIGHT

It is well known that small pineapple plants produce small fruits and large plants large fruits (3). In order to investigate this relation more precisely, a number of experiments were performed in which individual fruits were weighed at harvest time, and, in addition, leaves which were green and over a foot in

TABLE 1

FLOWER FORMATION ON 2-YEAR-OLD CABEZONA PINEAPPLE PLANTS AFTER TREATMENT WITH 0.5 MG. OF NAPHTHALENEACETIC ACID (NA) IN DIFFERENT AMOUNTS OF DILUENT. TREATMENTS ON OCTOBER 5, 1945. OBSERVATIONS ON DECEMBER 19, 1945

Solutions applied	Total mg. NA per plant	No. of plants treated	% flowering
50 cc. of 0.001%.....	0.5	26	81
5 cc. of 0.01%.....	0.5	33	76
3 drops of 1% in alc....	0.5	27	30
None.....	0	88	0*

* Forty days later, on January 29, 1946, 23% of the controls were in flower because the flowering season had arrived.

length on each plant were counted, dead leaves and small ones being omitted. The fruit weight included the weight of the crown.

Figure 6 shows the result of such a test in which five hundred fruit weights were correlated with leaf numbers. Each fruit is represented by a cross. The plants were grown on a dry hillside without irrigation or fertilizer. When 18 months old, they were treated with 0.25 mg. of NA per plant. All flowered, regardless of size, and produced fruits which were harvested at the end of August, about $4\frac{1}{2}$ months after treatment.

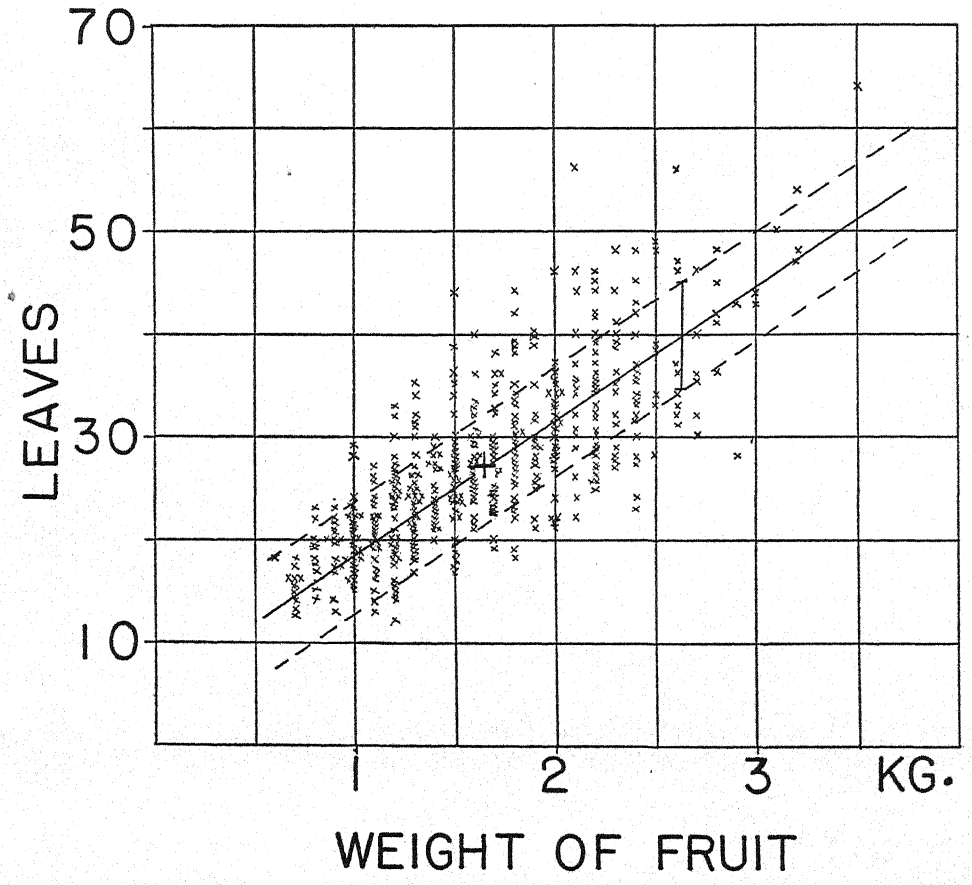


FIG. 6.—Relation between number of leaves per plant and weight of pineapple it produces. On April 15 plants were treated with 5 p.p.m. NA, when they were 18 months old. Harvest took place on August 24. Plants grew under adverse conditions. Slanting solid line is regression line; broken lines on both sides indicate standard error of estimate.

The correlation between leaf number and fruit size is statistically highly significant. A correlation coefficient of 0.76 was found; under the conditions of the experiment only 0.12 is required at the 1% level. The average fruit weight was 1.65 kg. and the average number of leaves 27.4. These values are represented by + in the diagram of figure 6. The regression coefficient was 11.5, which signifies that an increase of 11.5 leaves was accompanied by an increase of 1 kg. in fruit weight. From these data the regression line was constructed, which is represented as the slanting solid line in the diagram. Finally, the standard error of estimate was calculated, the value of which is indicated by the broken lines on each side of the regression line.

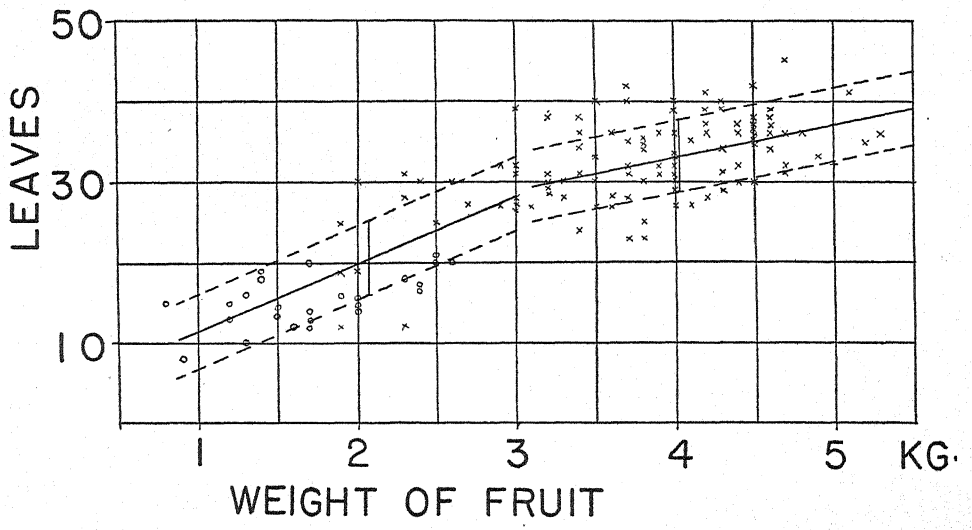


FIG. 7.—Relation between number of leaves per plant and weight of its fruit produced during normal season (crosses). Plants were 22 months old at time of harvest (May 15). Circles on left represent additional data for small plants treated with 10 p.p.m. NA when 14 months old and harvested at same time as naturally produced fruit. Plants grew under good conditions and may be compared with those of fig. 8.

Two additional correlation tests are given in figures 7 and 8. The plants of these tests were grown under excellent conditions on land where the plants referred to in figures 2 and 5 grew. The crosses of figure 7 show the relation between leaf number and fruit weight on nontreated plants. The plants were 22 months old when harvested during the middle of May. The circles, on the left, show the relation between leaf number and fruit weight of plants treated with 10 p.p.m. NA on November 25, and which were 20 months old at the time of harvest. The fruits of plants which had flowered naturally (crosses) represented only 8% of the total number of nontreated plants in that block. They were all large plants. Some additional plants (12%) were flowering at that time and produced fruits later in the season, while the remaining 76% did not flower during this season. Nearly 100% of the young, treated plants (circles) flowered (fig. 2).

Regression lines were constructed for fruits of 3 kg. or less and for fruits of more than 3 kg. The correlation coefficients (0.79 and 0.41, respectively) were statistically highly significant. The

regression lines and the standard error of estimate are shown in the diagrams in the same manner as in figure 6.

The diagram for figure 7 is comparable to that of figure 8 in that plants of the same block were used. They differ in that the plants of figure 8 were all treated with NA and harvested out of season (December 19). Treatment took place on July 21, when the plants were 22 months old. A comparison of figures 7 and 8 shows that NA-induced fruit, which was produced out of season, had exactly the same weight as that produced without treatment during the regular flowering season. For example, in order to produce a 4-kg. fruit during the season, an average number of thirty-two leaves is required (fig. 7). For NA-induced fruit, harvested out of season, we found precisely the same relation (fig. 8). Similarly, an average of twenty-eight leaves will give a fruit of 3 kg., regardless of whether the fruit was produced during the season and without treatment (fig. 7) or out of season and with treatment (fig. 8).

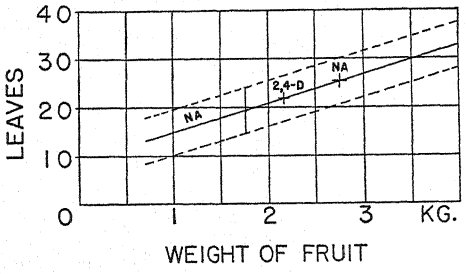


FIG. 8.—Relation between number of leaves per plant and weight of its fruit produced out of season. This diagram represents plants treated with NA (pooled data of 5 and 10 p.p.m.) on July 21, when they were 22 months old. Fruits harvested on December 19 and January 4 (pooled data). Cross marked NA shows average weight and average leaf number of NA-treated plants. Cross marked 2,4-D shows same for plants treated with 2,4-D with same concentrations and at same time as NA plants. Plants grew under good conditions in same block as those of fig. 7. Data of figures 7 and 8 are comparable (further data in table 2).

COMPARISON BETWEEN NA AND 2,4-D TREATMENTS

Previously (10) it was shown that with 2,4-dichlorophenoxyacetic acid (2,4-D) flowers may be induced with equal facility as with NA (fig. 5). Fruit production with 2,4-D is, however, somewhat delayed. For instance, in one test of 96 NA-treated plants 79% were harvested 5 months after treatment. Of 109 comparable plants treated with 2,4-D only, 56% had harvestable fruit 5 months after treatment. The remaining fruits were harvested 3 weeks later. Table 2 summarizes the results of flower induction with NA and 2,4-D. It is of interest to note that the smaller average weight of fruits of the 2,4-D plants, as compared with the NA plants, is accompanied by a proportionately smaller leaf number. This places the point (+) for average fruit weight and leaf number for 2,4-D plants exactly on the regression

TABLE 2

COMPARISON AT HARVEST TIME (DECEMBER 19) OF PINEAPPLE PLANTS OF CABEZONA VARIETY TREATED WITH NAPHTHALENE-ACETIC ACID (NA) AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D). POOLED DATA OF CONCENTRATIONS OF 5 AND 10 P.P.M. TREATMENTS ON JULY 21, 1945

	NA	2,4-D	Difference*
Av. no. of leaves.....	25.2 (100%)	21.8 (86.5%)	3.4 (13.5%)
Av. wt. of fruit (kg.)..	2.78 (100%)	2.18 (78.5%)	0.60 (21.5%)
Av. length of fruit stalk (cm.).....	34.9 (100%)	28.8 (82.5%)	6.1 (17.5%)
Degrees of freedom...	75	105	

* All differences statistically highly significant.

line for NA (fig. 8). This suggests that the lower average weight of 2,4-D fruit is caused by a lower leaf number of the 2,4-D plants as compared with those treated with NA. This, in turn, may be an effect of 2,4-D in preventing some leaves which were young at the time of treatment from reaching maturity. The interference of 2,4-D with normal leaf growth is well known (14), and certain relatively tender-leaved varieties of pineapple, such as the Smooth Cayenne, showed leaf deformations after treatments with low quantities of 2,4-D.

Discussion

Theoretical aspects

Which factors, external and internal, are responsible for the fact that under natural conditions flowers are initiated in the pineapple only in the winter season? Since the first flowers of the pineapple appear in December (fig. 1), flower initiation must have taken place early in November. When one examines which *external* factors are different in November as compared with the months preceding it, the decrease in length of day is especially notable. When length of day is plotted against the time of year, one finds for Puerto Rico that from April to August the length of day does not change greatly. For the middle of the month the daylengths are:[4] April, 12^h34^m; May, 12^h59^m; June, 13^h12^m; July, 13^h7^m; August, 12^h45^m. After August the length of day drops relatively sharply: September, 12^h14^m; October, 11^h44^m; November, 11^h17^m. After November it does not drop much farther: December, 11^h3^m; January, 11^h10^m. Then a rise sets in again: February, 11^h34^m; March, 12^h2^m.

October and November, therefore, must be critical months for the initiation of flowers in short-day plants. This is demonstrated by sugar-cane varieties which respond to changes in the length of day (7), and which in Puerto Rico always arrow with great regularity in the middle of November.

Preliminary data indicate that the pineapple also may be a short-day plant. Not only does its flowering period coincide with the period of the shortest daylength but it appears that an artificially increased daylength prevents natural flowering (but not chemically induced flowering). This was shown by rigging four 200-watt bulbs above forty-six pineapple plants. These were approaching their third season but had not yet flowered. By means of a time switch the lights were turned on from 7:00 P.M. until midnight. This light treatment was begun in August and was continued until January. After one month of extra illumination five plants were given 10 p.p.m. NA. Control plants, not receiving additional light, were treated similarly. Twenty days later five additional plants under the lights and five controls received 10 p.p.m. NA. Two months after the NA had been applied the plants receiving extra light flowered as well as the controls which had not received extra illumination. A long day, therefore, cannot prevent naphthaleneacetic acid from inducing flowers in the pineapple plant.

The same experiment yielded additional information. In February, well within the normal flowering season, 41% of the 223 control plants (not illuminated or treated chemically) had formed flowers. On the other hand, none of the 36 illuminated but nonchemically treated plants had then flowered. This suggests that the pineapple plant is a short-day plant. Further experiments are necessary, however, to ascertain this. It has

[4] Meteorological data obtained through courtesy of Dr. N. F. Childers of the Federal Experiment Station.

also been suggested that flower formation in the pineapple may be a response to relatively low temperature (6).

Which *internal* factors make the pineapple plant flower during the normal season? Since as little as 0.05 mg. per plant of naphthaleneacetic acid (fig. 2, the 1 p.p.m. curve) is in some cases sufficient to force a plant into flowering, it is tempting to attribute the normal flower production to increased hormone activity. Auxin analyses of the pineapple plant are necessary to provide further evidence. Such an analysis is feasible at present, because a convenient extraction method exists (12) for the determination of free auxin in plant materials.

It should not be overlooked, however, that the level of nutritional materials, such as carbohydrates and nitrogenous compounds, may undergo critical changes during the winter season which bring about flower induction. NIGHTINGALE's detailed observations of the Smooth Cayenne in Hawaii (6) have shown that plants deficient in nitrate and high in carbohydrates may fruit months earlier than others that contain more liberal nitrate reserves but a lower concentration of carbohydrates. It is not unlikely that a co-ordinated action of hormones of the auxin type and of nutritional factors determines the flowering of the pineapple plant, just as such action determines root formation in cuttings (11, 13).

PRACTICAL ASPECTS

By making use of diagrams such as presented in figures 6–8, the pineapple-grower can, with the aid of flower-inducing chemicals, control not only the time of harvest but the weight of his fruits as well. Since the vegetative growing-point changes into a floral apex after treatment, no additional foliage leaves are formed. Thus, for all practical purposes, the plant has to nourish its fruit with the leaves it has at the time of treatment.

When, for the particular conditions of a plantation, the leaf-number/fruit-weight diagram has been worked out, the weight of the fruit at harvest time can be predicted at the time of treatment. In addition, when a certain fruit size is desired, this can be obtained by treating the plants at a stage when they have a number of leaves which in the diagram corresponds to the desired fruit size. For example, when plants are growing under the conditions of figures 7 and 8, and a fruit weight of 3 kg. is desired, plants having between twenty-one and thirty-one leaves should be treated. When a smaller fruit size is desired, for instance, 2 kg., the plants should be treated when they have between sixteen and twenty-six leaves.

The experiments reported here with Cabezona pineapples demonstrate how controlled pineapple production throughout the year is possible. In these experiments, as demonstrated above, fruits weighed no less when produced out of season.

In order to have fruits of the desired size throughout the year, it is essential to have plants available of a size suitable for treatment throughout the year. This is possible for the Cabezona, which flowers with difficulty during the natural flowering season, because no undesirable flowering of immature plants takes place. A plant which flowers prematurely is lost for production.

In the opinion of the writer the low fruit weights which are reported when the Red Spanish variety is forced into flower outside the natural season are due to the fact that the plants treated are not sufficiently mature to support a large fruit. A continuous supply of plants

suitable for treatment is not available in this readily flowering variety because of loss due to the flowering of immature plants during the winter season.

For production throughout the year, without an accompanying fruit loss during the out-of-season months, it is therefore necessary to prevent undesired flowering of immature plants. This may be accomplished by using varieties such as the Cabezona which have a natural tendency to limited flowering, or by preventing flowering in the easy-flowering varieties by physiological means.

Summary

1. In the Cabezona variety of pineapple naphthaleneacetic acid (NA) can induce flowers in every month of the year (fig. 1).

2. Amounts of NA as small as 0.05 mg. per plant are capable of inducing flowers in a limited number of plants (1 p.p.m. curve of fig. 1), while 0.25 and 0.5 mg. per plant cause practically 100% flowering.

3. Fruit weight depends upon the size of the plant. The correlation between the number of leaves of a plant and the weight of the fruit it produces was determined (figs. 6–8).

4. Although 2,4-dichlorophenoxyacetic acid (2,4-D) has flower-inducing powers equal to NA (fig. 5), fruit induced by 2,4-D shows a lower average fruit weight as compared with fruit induced by NA (fig. 8 and table 2). This may be due to the adverse effect of 2,4-D on the development of young leaves.

5. Preliminary data indicate that the pineapple may be a short-day plant and that long-day conditions prevent natural flowering but not flowering induced by NA.

6. A method is outlined which makes fruit production in pineapples possible throughout the year without the loss of fruit weight now experienced by commercial growers when they force plants into flower out of season.

INSTITUTE OF TROPICAL AGRICULTURE
MAYAGÜEZ, PUERTO RICO

LITERATURE CITED

1. CLARK, H. E., and KERNS, K. R. Control of flowering with phytohormones. Science 95:536–537. 1942.
2. COOPER, W. C. Effect of growth substances on flowering of the pineapple under Florida conditions. Proc. Amer. Soc. Hort. Sci. 41:93–98. 1942.
3. COOPER, W. C., and REESE, P. R. Induced flowering of pineapples under Florida conditions. Proc. Florida State Hort. Soc. for 1941. Pp. 132–138.
4. FOSTER, M. B. A step ahead of mother nature. Natl. Hort. Mag. 22:127–129. 1943.
5. GRAY, R. W. La Distribución anual y geográfica de lluvia en Puerto Rico. Almanaque Agrícola para 1944 (Departamento de agricultura y comercia de P. R.). Pp. 47–58.
6. NIGHTINGALE, G. T. Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. BOT. GAZ. 103:409–456. 1942.
7. PARKER, M. W., and BORTHWICK, H. A. Day length and crop yields. U.S.D.A. Mis. Publ. 507. 1942.
8. RODRÍGUEZ, A. G. Influence of smoke and ethylene on the fruiting of the pineapple (*Ananas sativus*). Jour. Dept. Agr. Puerto Rico 16:1–18. 1932.
9. SNEDECOR, G. W. Statistical methods. Ames, Iowa. 1940.
10. VAN OVERBEEK, J. Flower formation in the pineapple plant as controlled by 2,4-D and naphthaleneacetic acid. Science 102:621. 1945.
11. VAN OVERBEEK, J., and GREGORY, L. E. A physiological separation of two factors necessary for the formation of roots on cuttings. Amer. Jour. Bot. 32:336–341. 1945.
12. VAN OVERBEEK, J., DÁVILA OLIVO, G., and SANTIAGO DE VÁZQUEZ, E. M. A rapid extraction method for free auxin and its application in geotropic reactions of bean seedlings and sugarcane nodes. BOT. GAZ. 106:440–451. 1945.
13. VAN OVERBEEK, J., GORDON, S. A., and GREGORY, L. E. An analysis of the function of the leaf in the process of root formation in cuttings. Amer. Jour. Bot. 33:100–107. 1946.
14. ZIMMERMAN, P. W. Formative influences of growth substances on plants. Cold Spring Harbor Symp. on Quant. Biology 10:152–157. 1942.

A COMPARISON OF HISTOLOGICAL RESPONSES OF BEAN PLANTS TO TRYPTOPHANE AND TO LOW CONCENTRATIONS OF INDOLEACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 574

MARY AILEEN MURRAY AND A. GERALDINE WHITING

Introduction

Tryptophane is one of the many chemical compounds which have been studied as growth-regulating substances. Its effects, however, have been questioned. WENT and THIMANN (9) considered tryptophane itself an inactive compound which is changed into an active substance in the plant. They stated that if tryptophane is applied to *Avena*, curvatures result in approximately 2 hours. During this period, they presumed, the tryptophane is converted into indoleacetic acid. The basis for this assumption is the known conversion of tryptophane into indoleacetic acid by bacteria and fungi. More recent chemical evidence on the problem has been presented by AVERY and BERGER (1), certain of whose experiments showed a significant discrepancy between the low yield (0.015%) of indoleacetic acid from pure tryptophane and the higher yield (0.022%) of indoleacetic acid from the natural precursor in plant tissues (crude corn). From these experiments and from other reports, AVERY and BERGER felt safe in concluding that tryptophane is not identical with the corn auxin precursor to which most of the activity is attributable but that tryptophane is a plant auxin precursor of a low degree of activity.

Both indoleacetic acid (5, 7) and tryptophane (6) have been studied in a series of experiments testing the growth-regulating effects of various compounds applied in lanolin to the cut surface of the decapitated second internode of young kidney-bean plants. KRAUS (6) stated that tissue responses of the bean stem to application of tryptophane are similar in some respects to response to indoleacetic acid but are very different in many other respects. These differences seem to invalidate the assumption that tryptophane acts as a growth-regulating substance only after conversion to indoleacetic acid. He compared responses obtained from applications of 3 and 1.5% indoleacetic acid with the reactions resulting from treatment with 2% tryptophane. Since recent studies (1) have shown that the conversion of tryptophane results in a relatively small percentage of indoleacetic acid, the concentrations of indoleacetic acid used by KRAUS are far above those which might be expected from conversion of 2% tryptophane. Consequently, the question arises: Would the response induced by 2% tryptophane resemble more closely that induced by a lower concentration of indoleacetic acid?

The purposes of this paper are (*a*) to report the results of histological studies with five concentrations of indoleacetic acid—0.2, 0.02, 0.002, 0.0002, and 0.00002%—and (*b*) to compare these histological responses with those described by KRAUS for tryptophane. The materials and the methods are the same as those used in earlier studies of indoleacetic acid (7) and of tryptophane (6).

Results

Following decapitation and treatment with indoleacetic acid, proliferation of many of the cells at the cut surface takes place. This applies especially to those cells which are parenchymatous or incompletely differentiated. As general proliferation progresses, the normal pattern of tissue organization is quickly lost. It is this growth which forms the tumor above the cut surface. Below the zone of general proliferation the pattern of responding tissues is similar to the normal tissue arrangement in the stem. It is perhaps in this region that the most characteristic features of the histological effects of the various growth-regulating substances are to be found, although the character of the tumor may likewise be distinctive. With increasing distances from the surface of application, the response becomes less pronounced, until a level is reached beyond which no effect is evident. The degree of reaction varies among individual plants and from bundle to bundle within the stem, major bundles showing the greatest degree of reaction. Environmental factors also influence the response.

From gross observations and from preliminary studies of histological material for the five concentrations of indoleacetic acid, it is evident that response is greatest with 0.2%, less with 0.02%, and only slight with 0.002%. The two lowest concentrations (0.0002 and 0.00002%) are apparently below the threshold of effectiveness for indoleacetic acid to induce histological changes or tumor formation under the conditions of this experiment (fig. 13*C*). Response to these two concentrations is the same as that which may result from decapitation and application of pure lanolin. The essential features of the histological responses to the three effective concentrations are summarized in table 1. Supplementary details and variations are reported in the following paragraphs.

At the cut surface the epidermal cells are injured (fig. 9), and those cells immediately adjacent may enlarge slightly and divide in radial, transverse, or tangential planes. This response is limited in extent and may occur in any one of the concentrations of indoleacetic acid or in control material. Below this level the epidermis shows the usual adjustment to increase in stem diameter. However, with the extreme enlargement at points of root formation, rupture of the epidermis may occur.

Response in the cortical parenchyma is correlated with the degree of concentration. With 0.2% indoleacetic acid the outer cortical parenchyma cells become conspicuously enlarged at the upper levels (fig. 7*A*). Cell division is infrequent. The inner parenchymatous cells, especially those directly adjoining the endodermis, become meristematic (figs. 2*A*, 3*A*) and, like it, form part of the capping tissues over root primordia (figs. 3*B*, 7*A*). Otherwise the derivatives mature as parenchyma. Cortical response is most pronounced in the upper 2 mm. of the stem tip and progressively decreases to the 3- or 4-mm. level. With the 0.02% concentration, cell enlargement is distinctly less (fig. 4*A*). There may be a few divisions in the inner cells, but they do not become highly meristematic. This response extends approximately 1 mm. from the cut surface, with only traces below this. By the 4th day derivatives are maturing as parenchyma (fig. 5*B*). In the lowest effective concentration (0.002%) there appears to be no response in the cortical parenchyma (fig. 4*B*).

The endodermis is the most highly re-

sponsive tissue. By the end of 24 hours meristematic activity is induced by all three concentrations which cause any response. With the highest concentration (0.2%) the endodermis shows an increasing number of tangential divisions by the 2d day (figs. 1*B*, 3*A*). By the 3d day it has become a relatively wide band of meristematic tissue (fig. 2*A*) with the initiation of root primordia under way at points of intense activity (fig. 3*B*). The zone of root formation and intense reaction extends to approximately 1 mm. below the cut surface. Lower than this the degree of meristematic activity decreases rapidly. Tangential divisions are fewer and the characteristic radial arrangement is more evident. Traces of response are found 5–6 mm. below the treated surface. Although the upper levels may remain meristematic in areas associated with root formation or vascularization (fig. 5*A*), evidence of maturation of the derivatives into parenchyma generally occurs by the third or fourth day. The formation of small vascular strands is initiated during the period of meristematic activity, and divisions in various planes result in patches of small cells. By the 3d or 4th day certain of the cells in the center of the patch may mature as reticulate tracheids, whereas peripheral cells remain meristematic (figs. 6*A*, 12*B*). The size and organization of these vascular strands increase until, in some stems, large bundles are formed (fig. 17*D*). Endodermal bundles are characteristic of the upper millimeter of the stem tip. Below this a few strands may end blindly in the endodermis, but others pass from the endodermis across the pericycle into the phloem.

Endodermal response at uppermost levels to the 0.02% concentration is similar to that below the zone of root formation with the highest concentration. Response is initiated by the end of 24 hours and consists mainly of tangential divisions which form a band of active tissue. At upper levels this band may be as much as eight cells deep over the major bundles of the stem (figs. 1*C*, 2*B*). At the depth of 1 mm. the number of endodermal derivatives decreases, and only traces of activity are found below 2 mm. Root primordia were not observed in any material treated with this concentration. By the 3d or 4th day derivatives begin to mature. This is made evident by the rounding-up of cells as parenchyma or by the occasional formation of reticulate tracheids and weakly organized vascular strands (figs. 5*B*, 7*B*, 13*A*). Vascularization follows the same course as in stems treated with the higher concentrations. The next lower concentration—0.002% —induces noticeable response only in the endodermis at uppermost levels. By the end of 24 hours tangential endodermal divisions are evident over two or three major bundles of the stem, frequently only along the sides of these bundles. By the 2d day the number of divisions may increase slightly (fig. 1*D*), after which there is little change (figs. 5*C*, 13*B*). A depth of 1 mm. seems to be the level of histological response farthest from the surface of application (figs. 4*B*, 6*C*). The derivatives mature as parenchyma (fig. 2*C*).

The pericycle responds in a limited manner. At the upper levels with the two higher concentrations, the cells enlarge, often proliferate slightly, and mature as parenchyma (figs. 3*A*, 4*A*, 7*A*, 9). At lower levels the pericyclic cells mature as the usual thick-walled fibers. The development as fibers is fairly well established at 3 mm. with the 0.2% concentration. With the two lower concentrations, well-formed fibers may be observed at less than the 1-mm. level (fig. 6*B*, *C*).

Crushed pericyclic cells were only rarely observed.

The primary phloem is nearly as sensitive as the endodermis, although the reaction is never as conspicuous. The initial stages of response are cell enlargement and an increasingly meristematic condition, which at the cut surface results in general proliferation (figs. 4*A*, *B*, 9). The greatest activity is found with the 0.2% concentration. The primary phloem parenchyma becomes highly meristematic and generally indistinguishable from active endodermal and ray tissues at those levels in which root formation takes place (fig. 2*A*). Below the root zone (somewhat below 1 mm.) the organization of tissue areas is re-established and a lesser degree of proliferation in the phloem is evident. By the 4th day the derivatives, except those in the root zone, are maturing as parenchyma or are differentiating as vascular elements. With the 0.02% concentration the effects in the phloem are similar to those with the higher concentration at levels below the zone of root formation. Enlargement and cellular proliferation were observed to levels 2 mm. deep. The extent of the histological response to the 0.002% concentration is general proliferation at the cut surface.

Vascularization of the primary phloem parenchyma commences with the organization of groups of small meristematic cells, similar to those found in the endodermis. With the 0.2% concentration some of these cells may differentiate as short reticulate tracheids, and the organization of a vascular strand may or may not follow. Poorly organized vascular strands were observed at the 0.5-mm. level (fig. 5*A*). They are better organized at lower levels (figs. 12*A*, 14*A*). As has already been noted, these vascular bundles may be in the endodermis at upper levels, with their course converging inward to pass across the pericycle, possibly at the 1-mm. level, where these cells are meristematic, and thence into the phloem (fig. 9). Within the phloem these vascular strands extend downward for 1 mm. or more. Many of these bundles terminate in direct inward connections through a phloem ray with the secondary phloem, cambium, and secondary xylem of the stele (figs. 10*A*, *B*, 11); other bundles may end blindly in the phloem. Branching of these vascular bundles may occur, especially toward the upper levels, where they pass outward through the pericycle into the endodermis. The origin, structure, and course of vascularization with the 0.02% concentration are similar.

The secondary phloem is relatively unaffected by the various concentrations. At levels just below the cut surface, the parenchyma and immature elements may proliferate (fig. 4*A*). Response below this general proliferation is limited to parenchymatous elements which may show a slight degree of cell enlargement (fig. 2*B*).

The cambium, like other undifferentiated tissues, tends to proliferate at the cut surface. Below the level of application the degree of activity is greatest with the 0.2% concentration and less with 0.02%; with 0.002% it is no greater than that found in stems decapitated and treated with pure lanolin. The zone of undifferentiated cells about the cambium becomes proportionately wider. Maturation of these undifferentiated cells indicates that the larger number of derivatives is cut off toward the xylem.

Xylem vessels and tracheids lignified previous to treatment remain relatively unchanged. However, xylem parenchyma and incompletely mature elements (fig. 4*A*) at the cut surface proliferate.

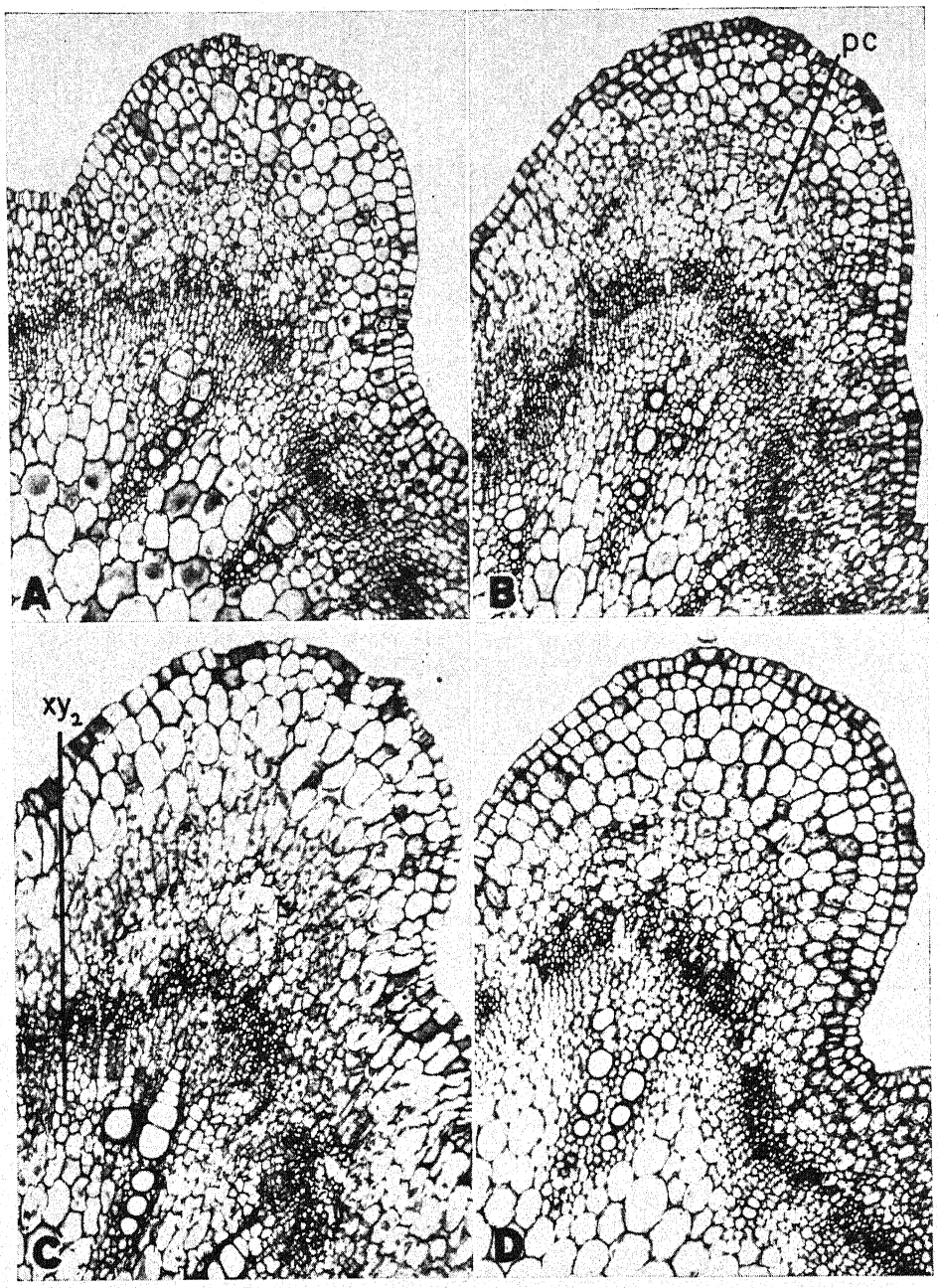


FIG. 1.—Transections of decapitated stems, 0.5 mm. below cut surface: *pc*, pericycle; xy_2, secondary xylem. *A*, 1 day after treatment with 0.2% indoleacetic acid. Cells of inner cortical parenchyma and endodermis dividing; primary phloem parenchyma meristematic; increased activity in cambium. *B*, 2 days, 0.2%. Endodermis forming meristematic band; pericycle and primary phloem parenchyma cells enlarged and meristematic; secondary xylem tracheids differentiating. *C*, 2 days, 0.02%. Similar to *B* but less in extent. *D*, 2 days, 0.002%. Some tangential divisions in endodermis; otherwise little response.

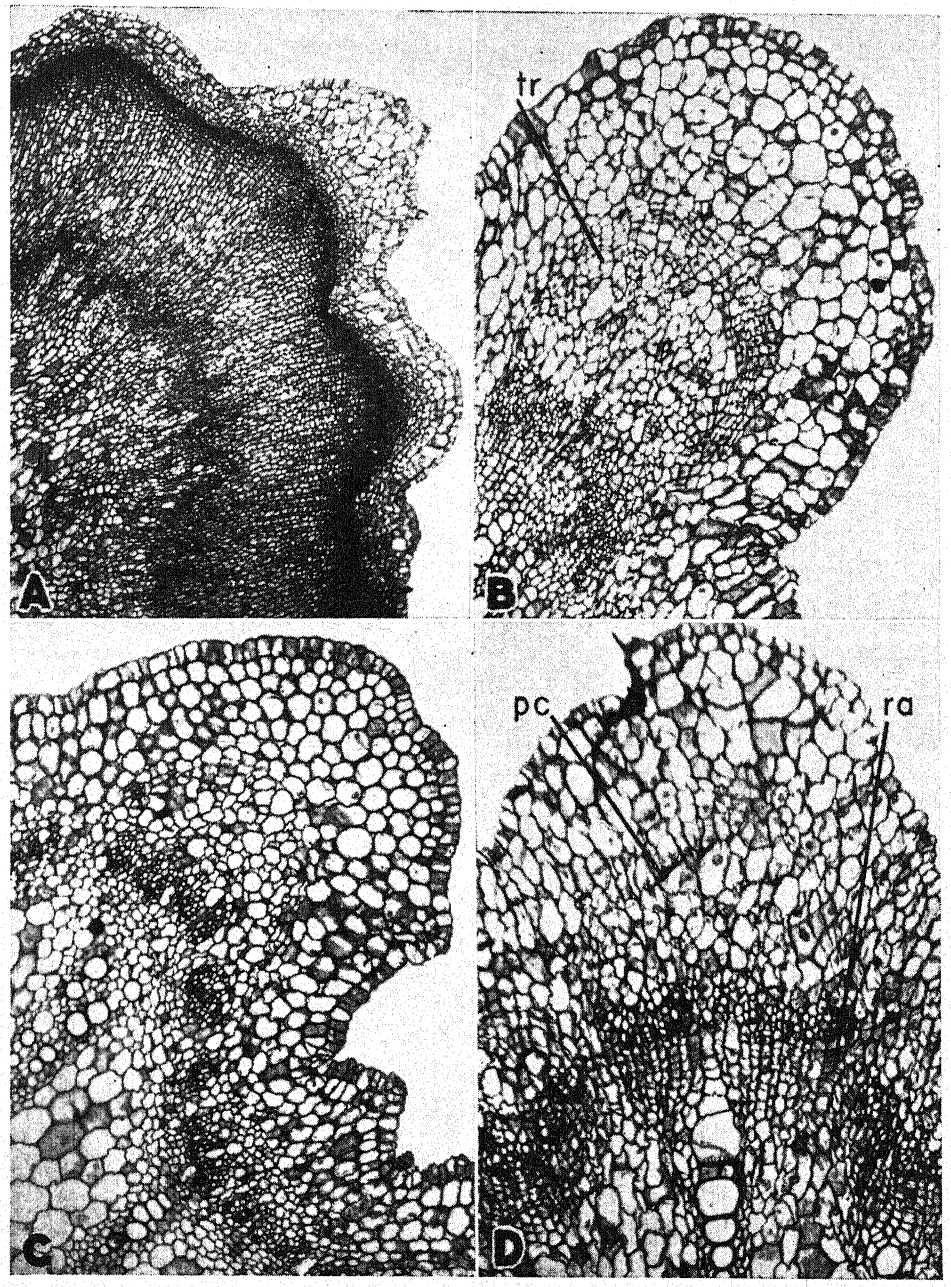


FIG. 2.—Transections of stems, 3 days after treatment, 0.5 mm. below cut surface: *tr*, tracheid; *ra*, ray. *A*, 0.2% indoleacetic acid. Highly meristematic band of proliferated endodermis, inner cortical parenchyma, pericycle, and primary phloem and ray parenchyma. Proliferation of pith parenchyma commencing centrad to protoxylem points. *B*, 0.02%. Maturation commencing with differentiation of some tracheids; other cells maturing as parenchyma. *C*, 0.002%. Response no greater than at 2 days. *D*, 2% tryptophane. Early stage in proliferation of endodermis; pericycle crushed (*pc*); little response in phloem; interfascicular cambium developing in ray (*ra*).

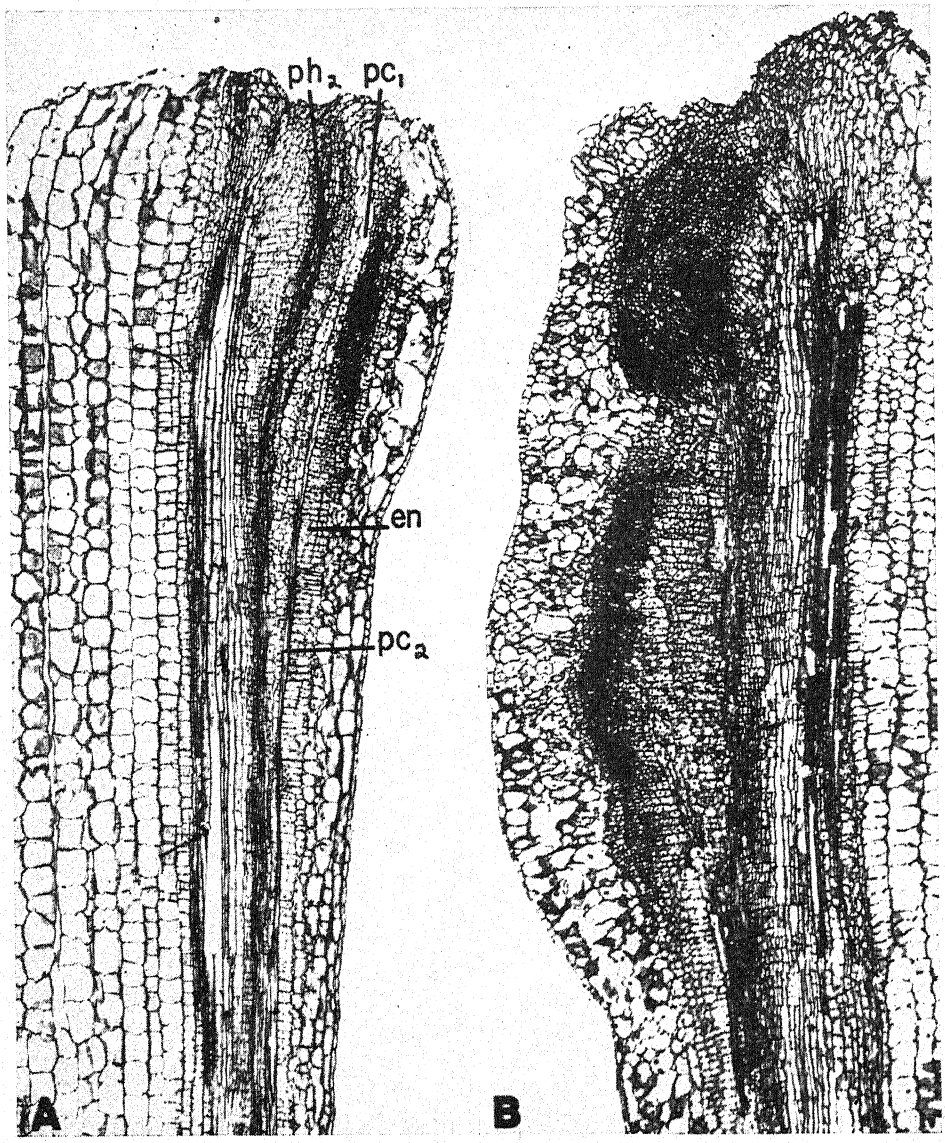


FIG. 3.—Longisections, 0.2% indoleacetic acid: *en*, endodermis; ph_2, secondary phloem. *A*, 2 days. Cells of outer cortical parenchyma greatly enlarged, inner ones proliferating; at upper levels in endodermis initiation of root primordium, at lower levels cell elongation and tangential divisions; at upper levels pericyclic cells (pc_1) enlarged and proliferating, at lower levels pericyclic fibers (pc_2); marked activity in primary phloem and cambium at upper levels. *B*, 3 days. Response similar but greater in degree than at 2 days; several root primordia evident; cambial derivatives differentiated as secondary xylem tracheids.

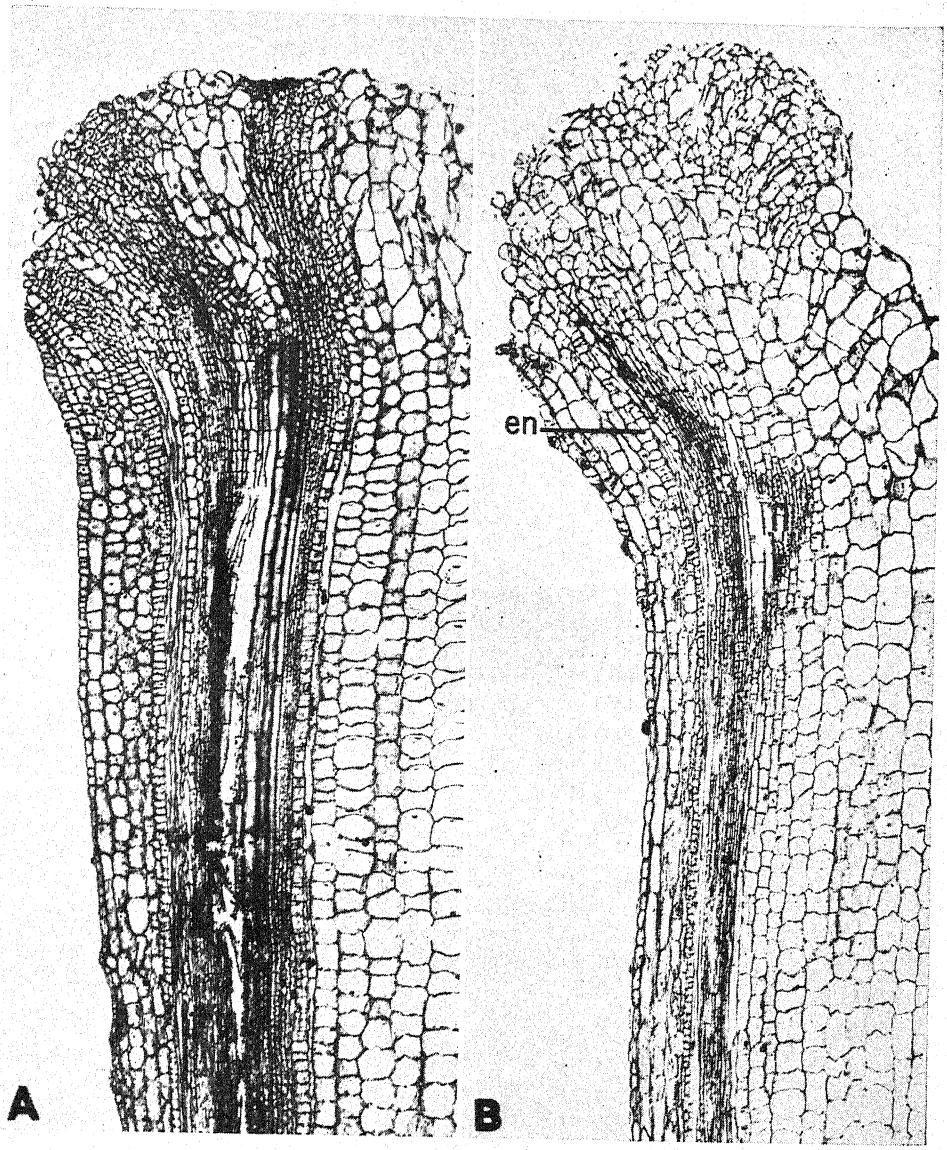


FIG. 4.—Longisections, 3 days after treatment. *A*, 0.02% indoleacetic acid. Across cut surface general proliferation of tissues except epidermis; conspicuously large cells are proliferated immature tracheal elements; below cut surface slight proliferation in cortical parenchyma, endodermis, pericycle, and primary phloem parenchyma. *B*, 0.002%. Similar proliferation of tissues at surface of application. Section through side of bundle showing slight proliferation in endodermis. (Compare with lateral position of proliferated endodermal cells in fig. 1*D*.)

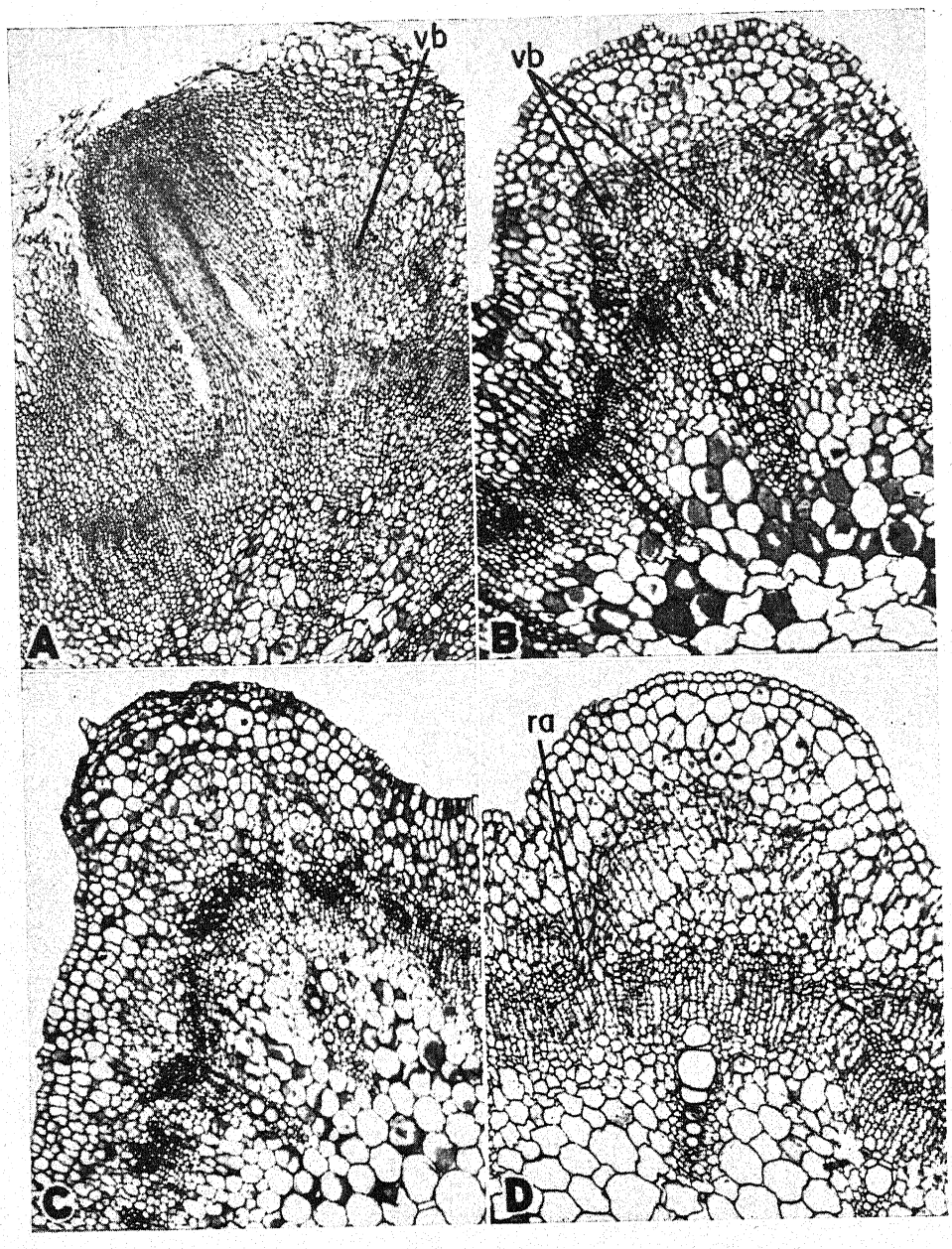


FIG. 5.—Transections 0.5 mm. below cut surface, 4 days after treatment: *vb*, vascular bundle. *A*, 0.2% indoleacetic acid. Root primordium and associated tissues meristematic, other tissues maturing; formation of vascular strands (*vb*) in primary phloem parenchyma commencing; numerous scattered secondary tracheids differentiated in xylem; proliferated ray and pith parenchyma differentiating tracheids and vascular strands. *B*, 0.02%. Similar to 3 days, more mature; vascularization (*vb*) of endodermis and phloem, strands poorly organized. *C*, 0.002%. Similar to 2-day response. *D*, 2% tryptophane. Continued proliferation in cortical parenchyma and endodermis; primary phloem parenchyma slightly meristematic. Note initiation of cambial strands in endodermis along sides of bundles and in meristematic ray (*ra*).

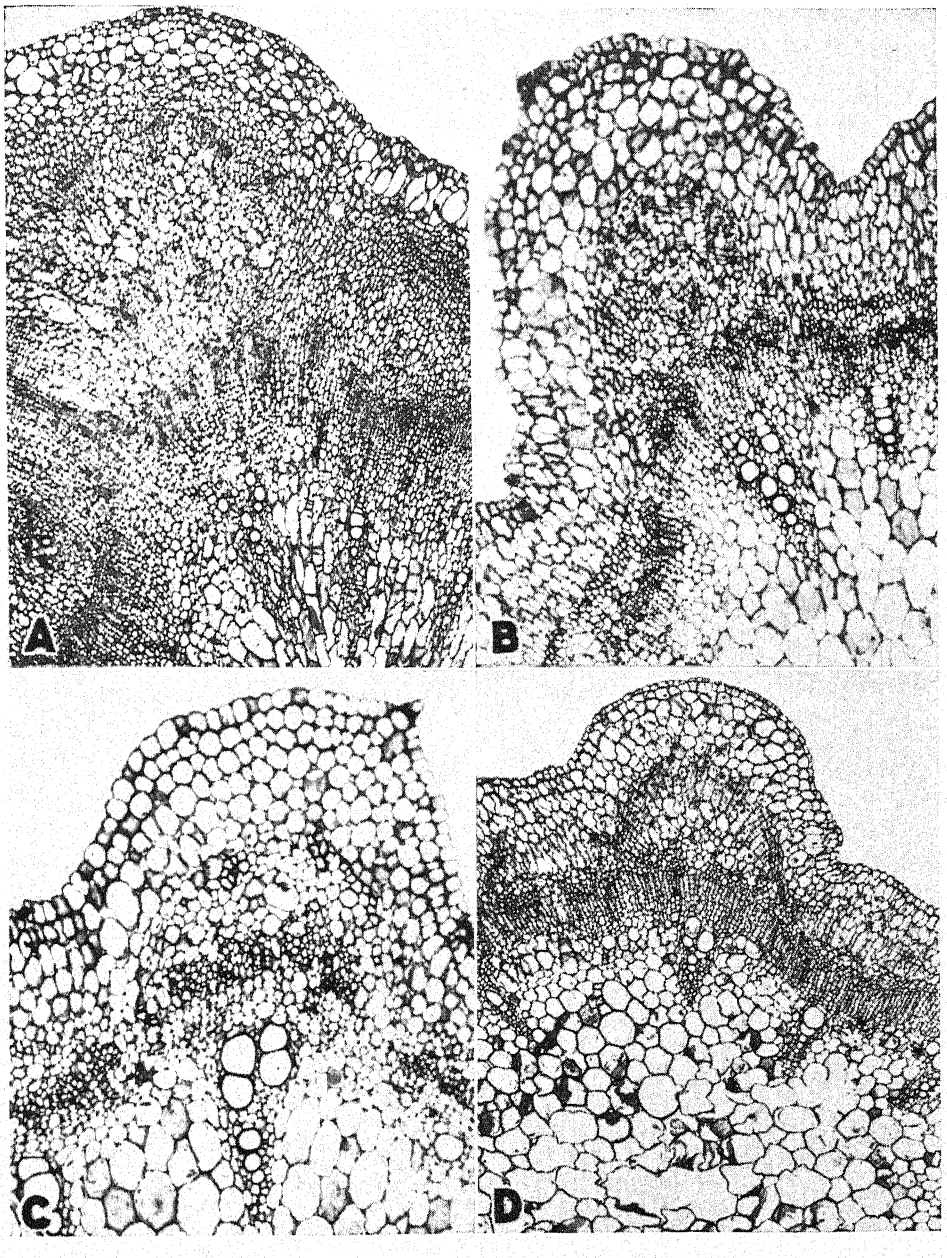


FIG. 6.—Transections 1 mm. below cut surface, 4 days after treatment. *A*, 0.2% indoleacetic acid; *B*, 0.02%; *C*, 0.002%; same stems respectively as in fig. 5. *D*, 2% tryptophane. Degree of response with each treatment somewhat less at this lower level.

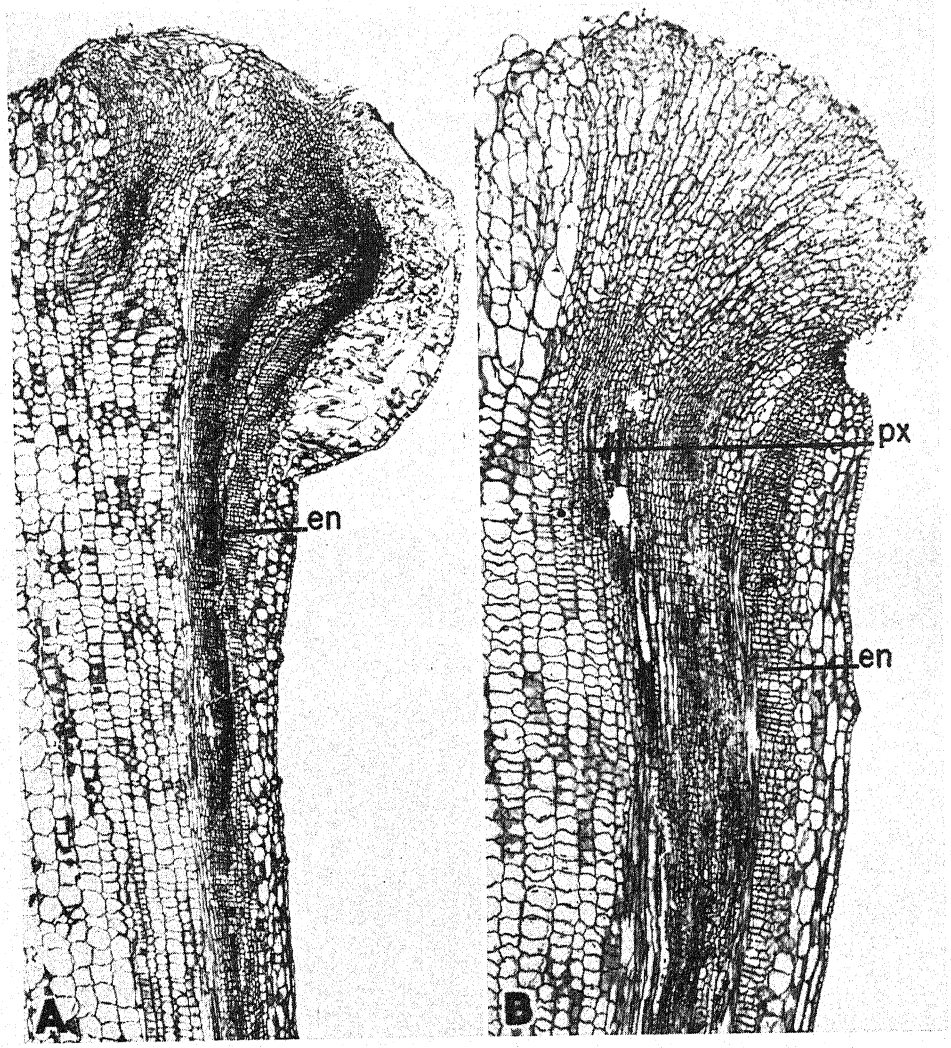


FIG. 7.—Longisections, 4 days after treatment: *px*, protoxylem. *A*, 0.2% indoleacetic acid. Section in ray showing relationship of tissues in development of root primordium; proliferation of marginal pith at upper level. *B*, 0.02%, early stages in development of tumor above cut surface. Note active proliferation of central pith; proliferated tissues below cut surface generally maturing.

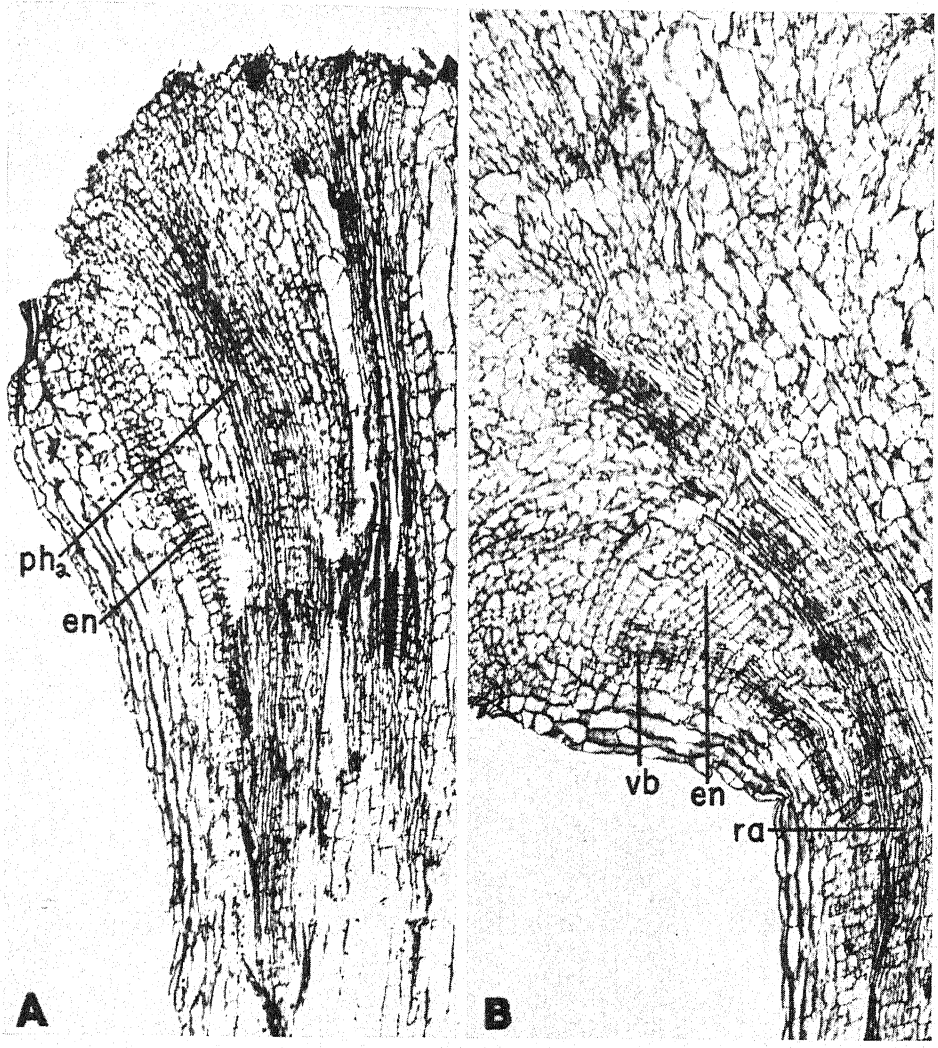


FIG. 8.—Longisections of stems treated with 2% tryptophane. *A*, 4 days after treatment. Early stage of tumor; active proliferation of tissues at lower levels; pith disintegrating. Compare with more advanced response in fig. 7*B*. *B*, 7 days. More advanced stage in formation of tumor; greatly proliferated endodermis, derivatives differentiated as vascular strand (*vb*), elements of which connect with stele through derivatives of ray (*ra*).

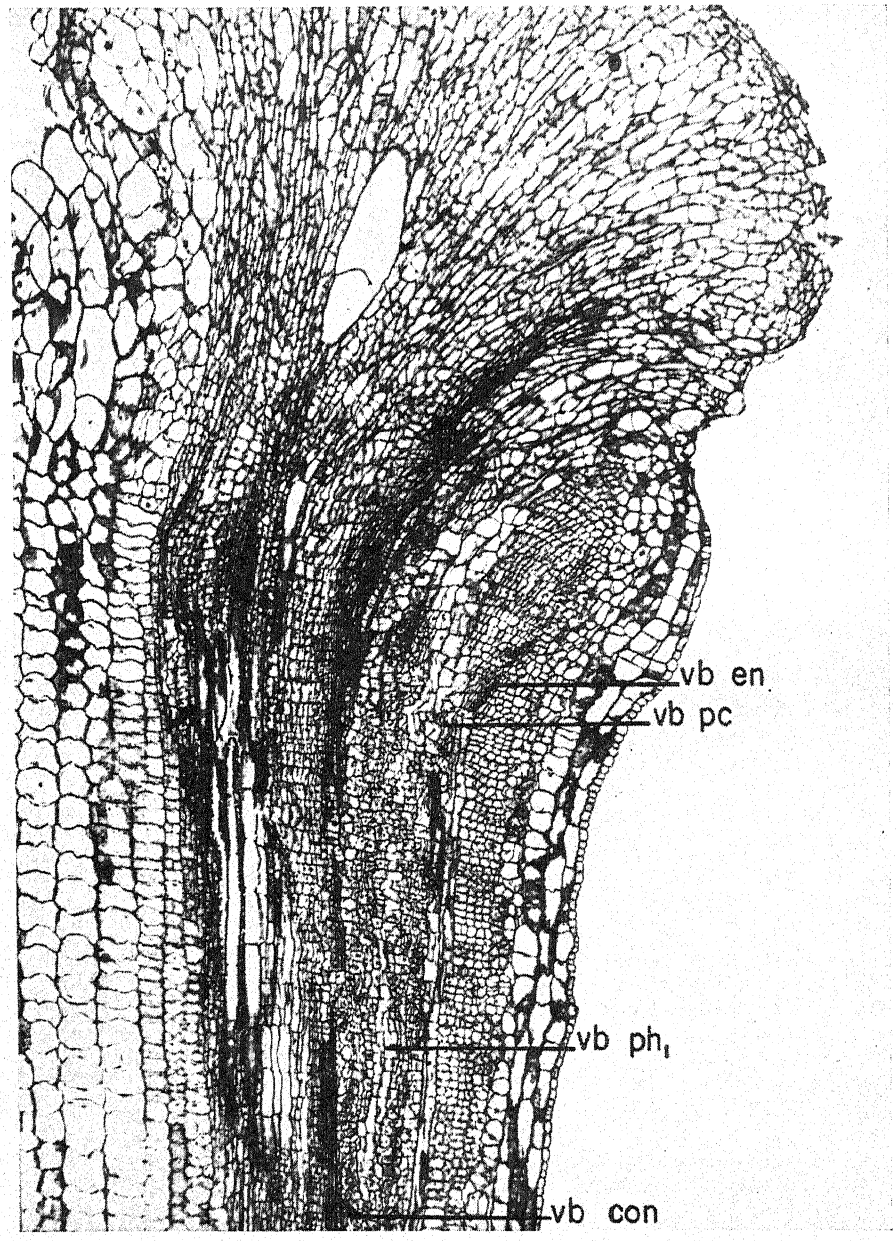


FIG. 9.—Longisection 6 days after treatment with 0.2% indoleacetic acid, showing course of vascularization: *vb con*, vascular connection; ph_1, primary phloem. At upper levels vascular bundle differentiated in derivatives of endodermis (*vb en*); at 1 mm. in proliferated pericyclic cells (*vb pc*); at lower levels in derivatives of primary phloem parenchyma (*vb* ph_1); at 2.5 mm. vascular bundle connects directly inward (*vb con*). See fig. 10*A*.

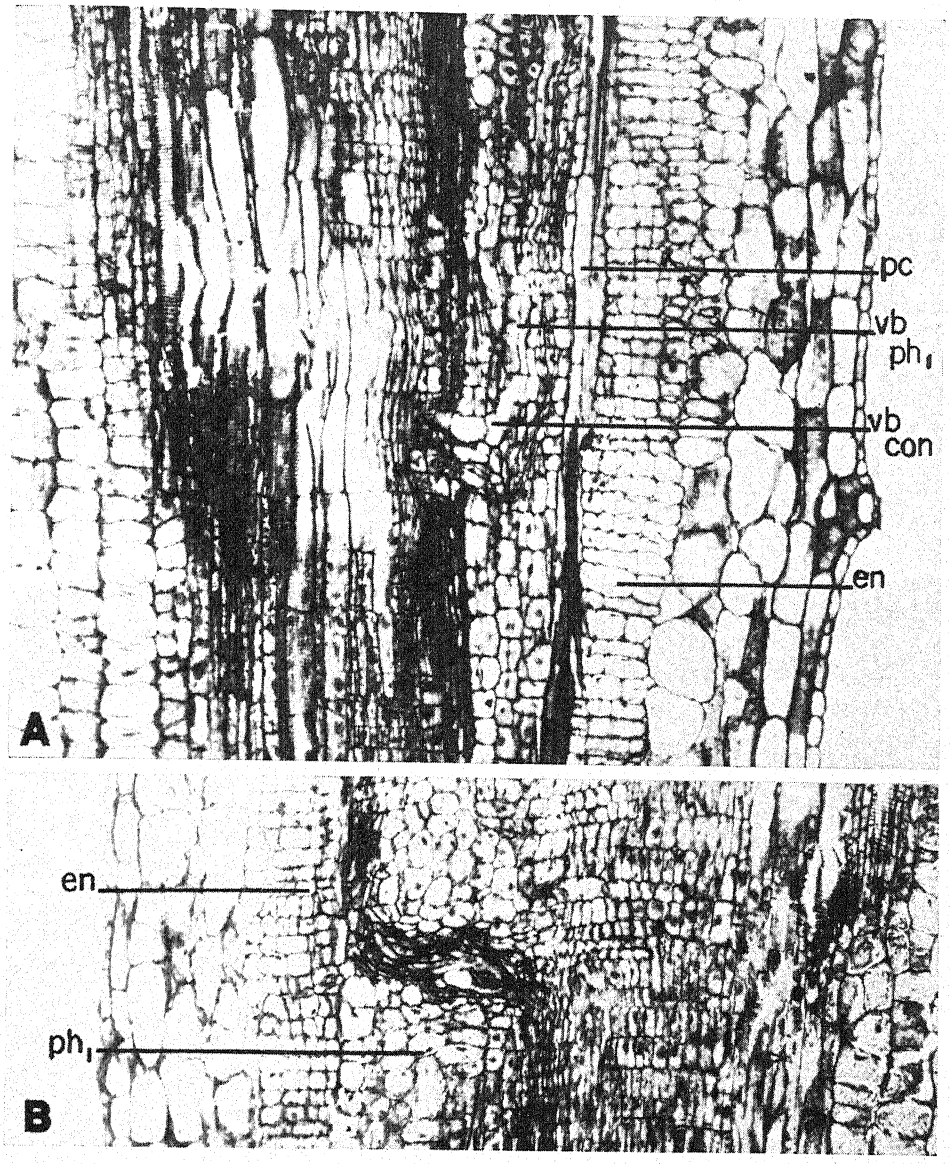


FIG. 10.—Longisections, 0.2% indoleacetic acid. *A*, 6 days after treatment, same stem as fig. 9. Cellular detail of connection (*vb con*) between vascular tissues of primary phloem bundle (*vb* ph_1) and secondary phloem, cambium, and secondary xylem of stele. *B*, 18 days after treatment. Detail of similar connection in another stem.

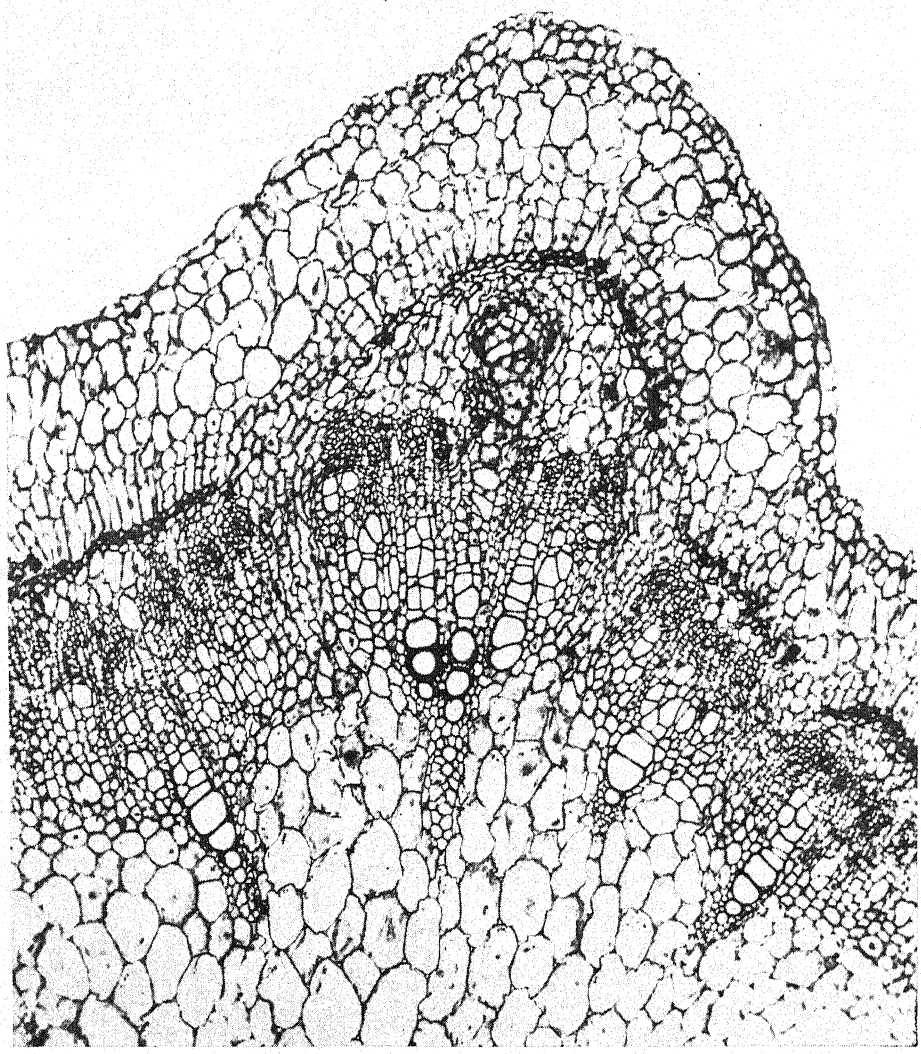


FIG. 11.—Transection 9 days after treatment with 0.2% indoleacetic acid, 2.5 mm. below cut surface. Same type of vascular connection as in fig. 10*A*. Endodermal derivatives matured as parenchyma; scattered tracheids in wide band of secondary xylem; medullary and xylem rays conspicuous.

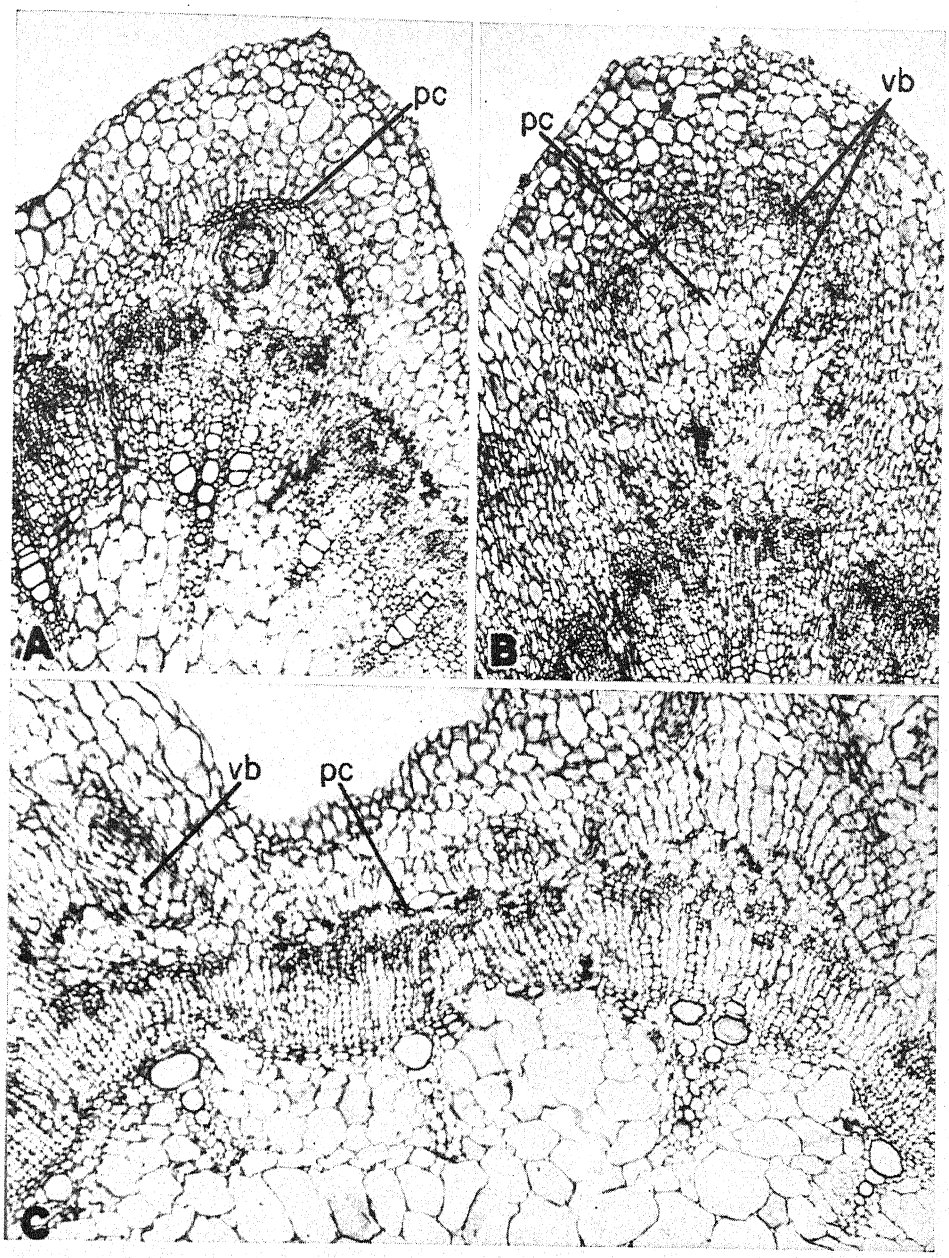


FIG. 12.—Transections, 9 days after treatment. *A*, 0.2% indoleacetic acid, 2 mm. below cut surface, same sector of stem as fig. 11. Clearly organized bundle in primary phloem parenchyma. *B*, same stem as *A*, 1 mm. below cut surface. Branches (*vb*) of same bundle, at this level in endodermis and primary phloem. *C*, 2% tryptophane, 1 mm. below cut surface. Vascularization (*vb*) in proliferated endodermis along flanks of bundles; no proliferation or vascularization in phloem or pith.

The greatest response is found in the differentiation of the cambial derivatives formed after treatment. At the highest concentration these derivatives form a proportionately wide band of short, reticulately thickened tracheids intermingled with occasional vessels and numerous ray cells (fig. 11). At 0.02% this band is similar but less wide (fig. 1*C*), and at 0.002% only traces of a tracheid layer are evident. The total downward extent of this response is difficult to determine as it blends with the secondary growth of unaffected portions of the stem.

On the basis of behavior a ray may be divided into an outer portion flanking the phloem and a portion flanking the xylem. With the 0.2% concentration the outer portion becomes an active part of the band of meristematic tissues in the upper 2 mm. of the stem tip (fig. 2*A*). The importance of the ray in the formation of root primordia has already been described in detail (4). Below the level of root formation the outer portion of the ray is very much less active, and its derivatives mature as parenchyma. An interfascicular cambium may or may not be formed. The response to 0.02% indoleacetic acid is similar to that below the root zone for the higher concentration. The lowest effective concentration (0.002%) induces no marked histological change in the ray.

The inner portion of the ray is associated with the pith, and the two may be described together. With both the 0.2% and the 0.02% concentrations (figs. 2*A*, 6*A*), response is delayed and proliferation is not initiated until the 3d or 4th day. Both tissues remain inactive with the 0.002% concentration. Large parenchymatous cells of the inner portion of the ray and of the marginal pith (pith abutting the rays or that centrad to the protoxylem points) may divide several times. The derivatives mature as parenchyma or as reticulate tracheids, or they remain meristematic to form vascular strands. The intensity of this reaction is greater at the 0.2% concentration, but with both concentrations the response is confined to the uppermost levels. It reaches slightly deeper than 1 mm. below the cut surface with 0.2% indoleacetic acid (fig. 15*A*) and less than 1 mm. for the other concentration. The central pith persists as a functional tissue. At the cut surface it proliferates but retains its parenchymatous character (fig. 15*B*).

The upward thrust of proliferating tissues to form the tumor above the cut surface commences by the 3d day (figs. 3*B*, 4*A*, *B*). Tissues from the pericycle outward participate but little in the growth of the tumor (figs. 7*B*, 15*B*). Proliferation above the vascular elements is greatest during the early stages, and derivatives form the peripheral portion of the tumor (figs. 9, 15*B*). In mature stages of the relatively small, dome-shaped tumor formed in response to 0.02% indoleacetic acid, proliferated pith forms the central portion (fig. 15*B*). With 0.2% the very large, cone-shaped tumor is formed almost entirely of proliferated pith (fig. 15*A*). The heavy marginal band of vascular tissue (fig. 17*A*) is connected with both the endodermal vascular system and the central stelar structure (fig. 17*A*, *B*, *C*, *D*). The anastomosing vascular strands in the center extend downward and connect directly with vascular strands arising in the pith (fig. 15*A*). With the lower concentration (0.02%), there is little vascularization and the tumor is mainly parenchymatous in composition. With the other three concentrations (0.002, 0.0002, and 0.00002%), the form and structure of the growth above the cut surface is similar to

those tumors which may be formed after decapitation and the application of pure lanolin (fig. 16*A*, *B*).

Discussion

A comparison of responses reported in this paper with those described earlier by KRAUS, BROWN, and HAMNER (7) clearly indicates that the four highest concentrations of indoleacetic acid (3.0, 0.2, 0.02, and 0.002%) induce responses which are similar in character and differ essentially only in degree. Successive decrements in concentration result in a series of decreasing expressions of response. Likewise, decapitated stems treated with higher concentrations of indoleacetic acid show a series of decreasing reactions at successive levels below the treated surface. Comparison of these two series shows that responses induced at successively lower levels in a stem tip treated with a high concentration are similar to responses at higher levels induced by treatment with the lower concentrations. The similarity between these two series suggests dependence of expression of response upon a concentration gradient of indoleacetic acid. On this basis, the absence of root primordia following application of lower concentrations should be expected rather than considered a striking dissimilarity. Apparently a certain intensity or threshold of reaction must be reached before development of adventitious roots occurs. With a concentration of 3% indoleacetic acid, this intensity is maintained a distance of several millimeters below the cut surface, and numerous root primordia are formed. When a stem tip is treated with 0.2% indoleacetic acid, fewer root primordia are developed, they are found within approximately the first millimeter below the cut surface, and the reaction within this limit is comparable with that at the base of the root zone with 3% indoleacetic acid. No root primordia are formed with the application of 0.02% indoleacetic acid. The maximum response is comparable with levels immediately below the root zone with applications of 0.2% indoleacetic acid. A concentration of 0.002% indoleacetic acid induces mere traces of response even at the surface of application. In appearance these responses are similar to the responses at the lowest levels with higher concentrations. The response of decapitated bean plants to treatment with various concentrations of indoleacetic acid follows a consistent pattern and varies only in degree.

BEAL (4) has observed that, for the occurrence of a response, a certain concentration gradient of the indoleacetic acid must be established downward from the treated surface. He found, however, that the nutritional status of the tissues was closely associated with this gradient. Treated segments placed on nutrient agar lacking either sugar or nitrogen showed no gross enlargement or proliferation. Other writers (2, 3, 8) have also demonstrated very close relationship between the carbohydrate content of the tissues and the effectiveness of a given growth substance. On this basis, it is conceivable that a plant with limited supplies of carbohydrates and treated with a high concentration of indoleacetic acid could simulate the histological response of a plant containing carbohydrates in greater amount but treated with a low concentration of indoleacetic acid. It is evident that a comparison of concentrations and degrees of response can be made only by the use of plants which are uniform, or nearly so, in their growth status and in their chemical composition under identical or closely similar environments.

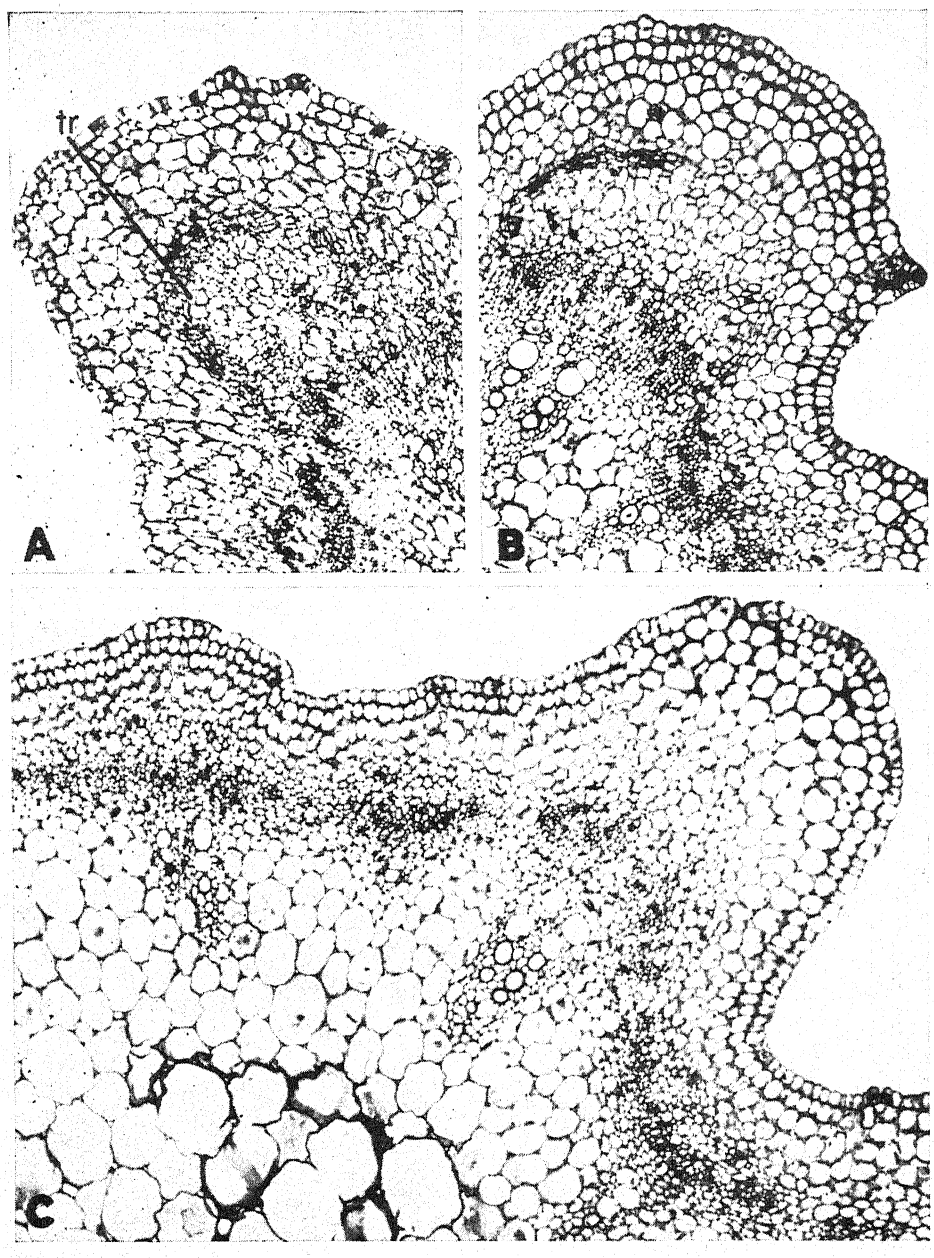


FIG. 13.—Transections 9 days after treatment. *A*, 0.02% indoleacetic acid, 1 mm. below cut surface. Proliferated cells in endodermis, pericycle, and primary phloem matured as parenchyma; a few scattered tracheids (*tr*) in small, poorly organized vascular bundles. *B*, 0.002%, 0.5 mm. Extent of response unchanged from 3 days. *C*, 0.0002%, 0.5 mm. Stem tissues showing no response; maturation proceeding as in untreated material.

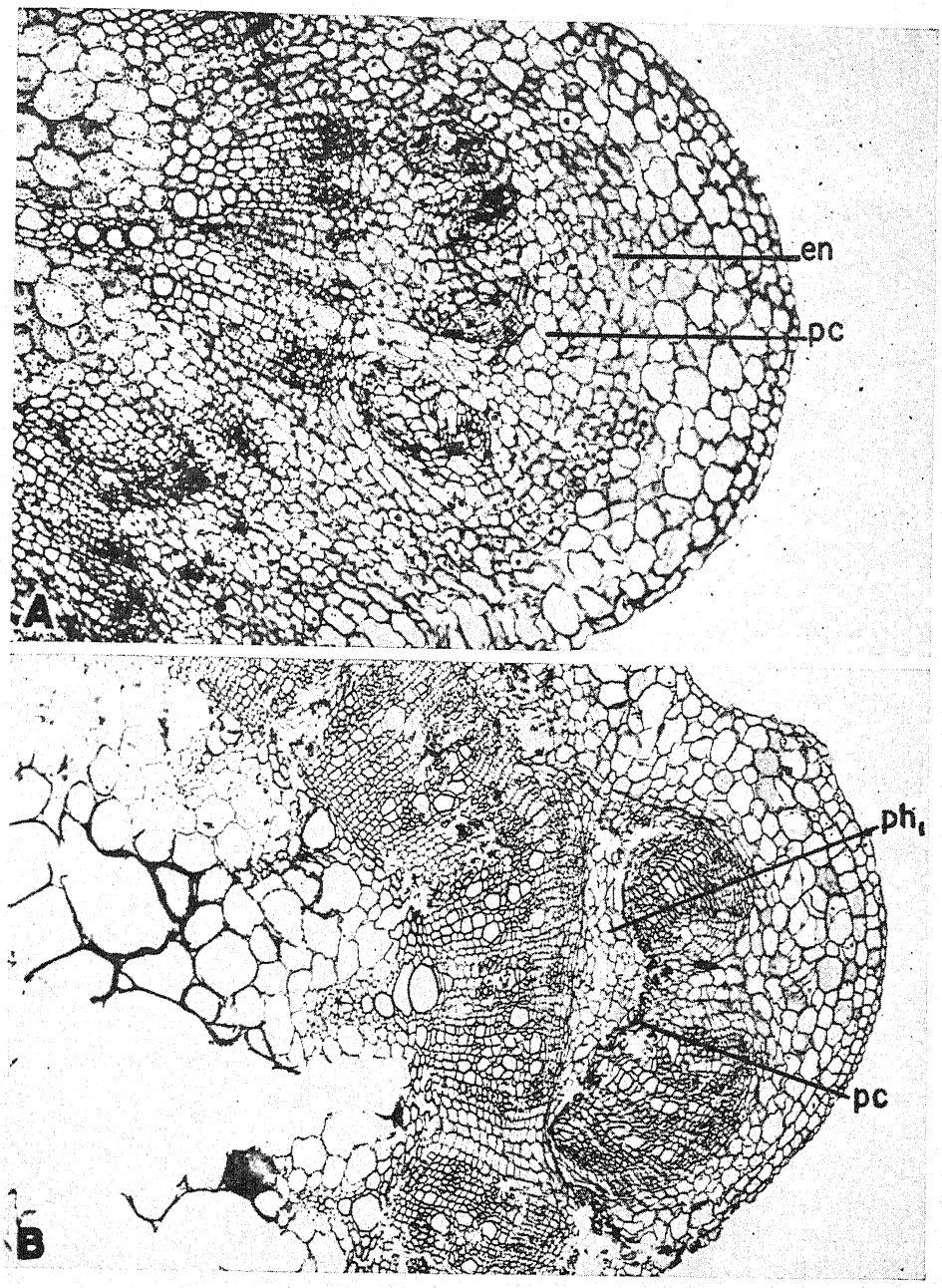


FIG. 14.—Transections of older stages showing position and character of vascularization. *A*, 14 days after treatment with 0.2% indoleacetic acid, 1.5 mm. below cut surface. Development of moderately large vascular bundles from derivatives of primary phloem parenchyma; proliferated endodermis maturing as parenchyma. *B*, 18 days after treatment with 2% tryptophane, at approximately 2 mm. Strongly developed bundles from derivatives of endodermis; no response in primary phloem parenchyma (ph_1). Compare compactly organized zone of secondary xylem with that in fig. 11. Disintegration of central pith characteristic for tryptophane.

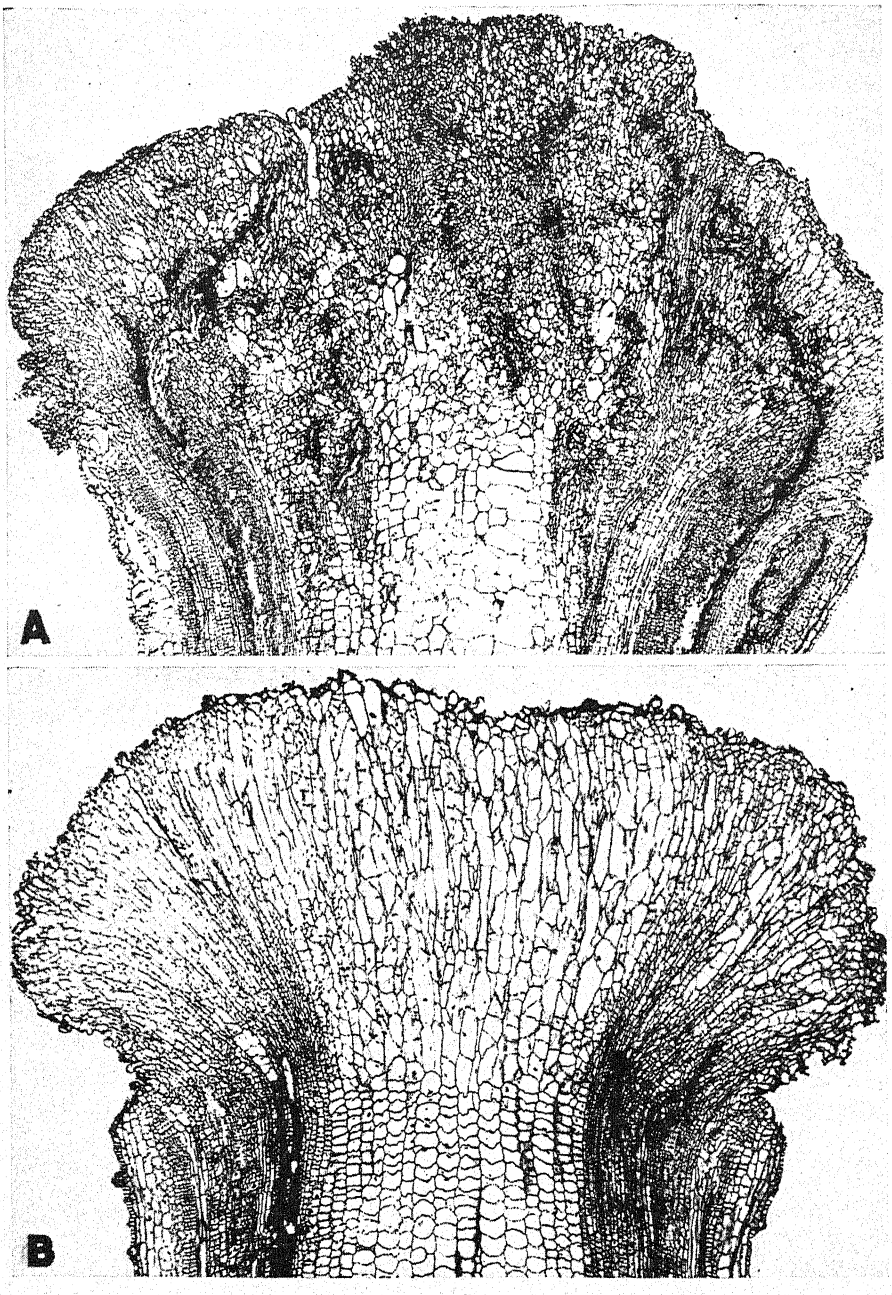


FIG. 15.—Longisections showing development of tumor above cut surface, 18 days after treatment. *A*, 0.2% indoleacetic acid. Cone-shaped tumor, largely derived from proliferation of pith; vascular tissues contributing to marginal area; complex system of vascular bundles showing connections with vascular cylinder of stem and vascular bundles proliferated in rays and pith. *B*, 0.02%. Smaller parenchymatous tumor, showing participation of pith as well as vascular tissues.

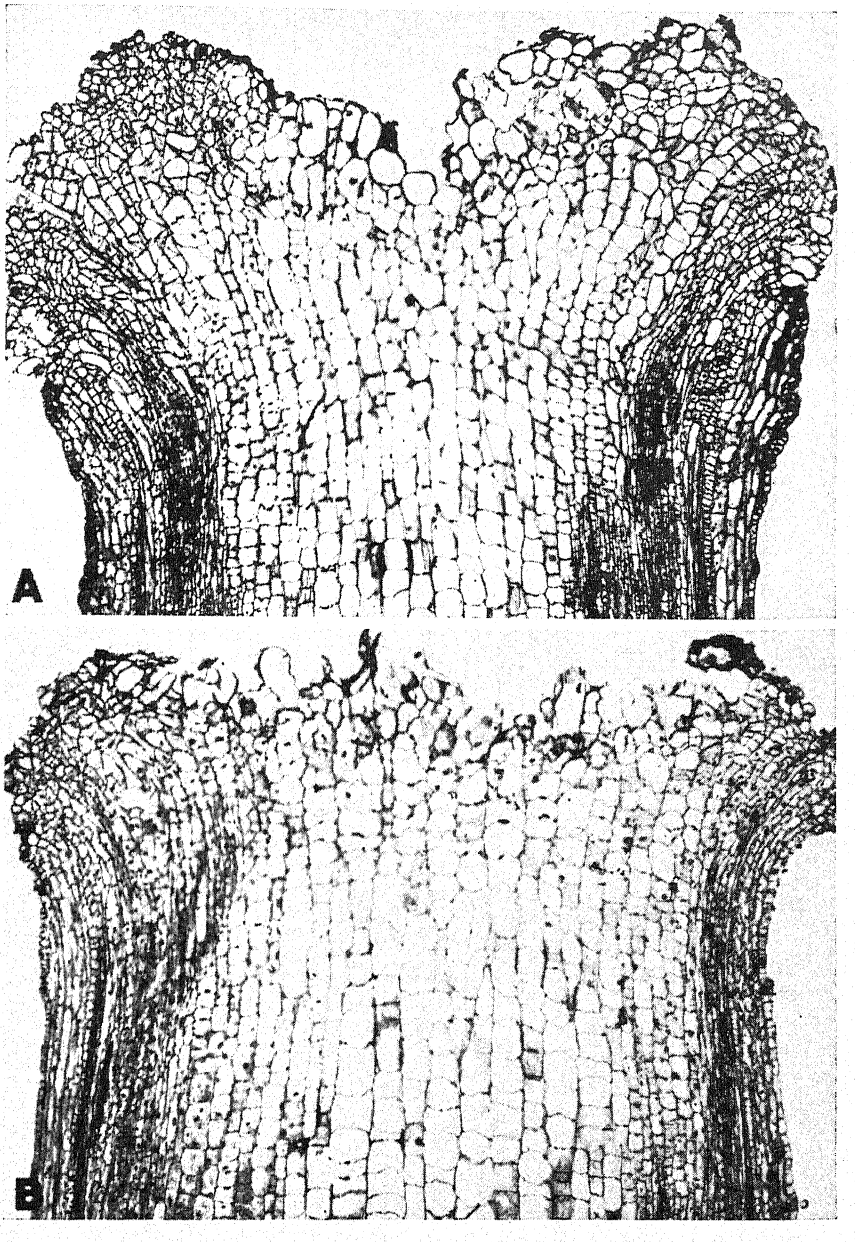


FIG. 16.—Longisections of stems treated with very low concentrations of indoleacetic acid. Limited proliferation at cut surface comparable with response in control material. *A*, 0.002%, 9 days after treatment. *B*, 0.0002%, 18 days after treatment.

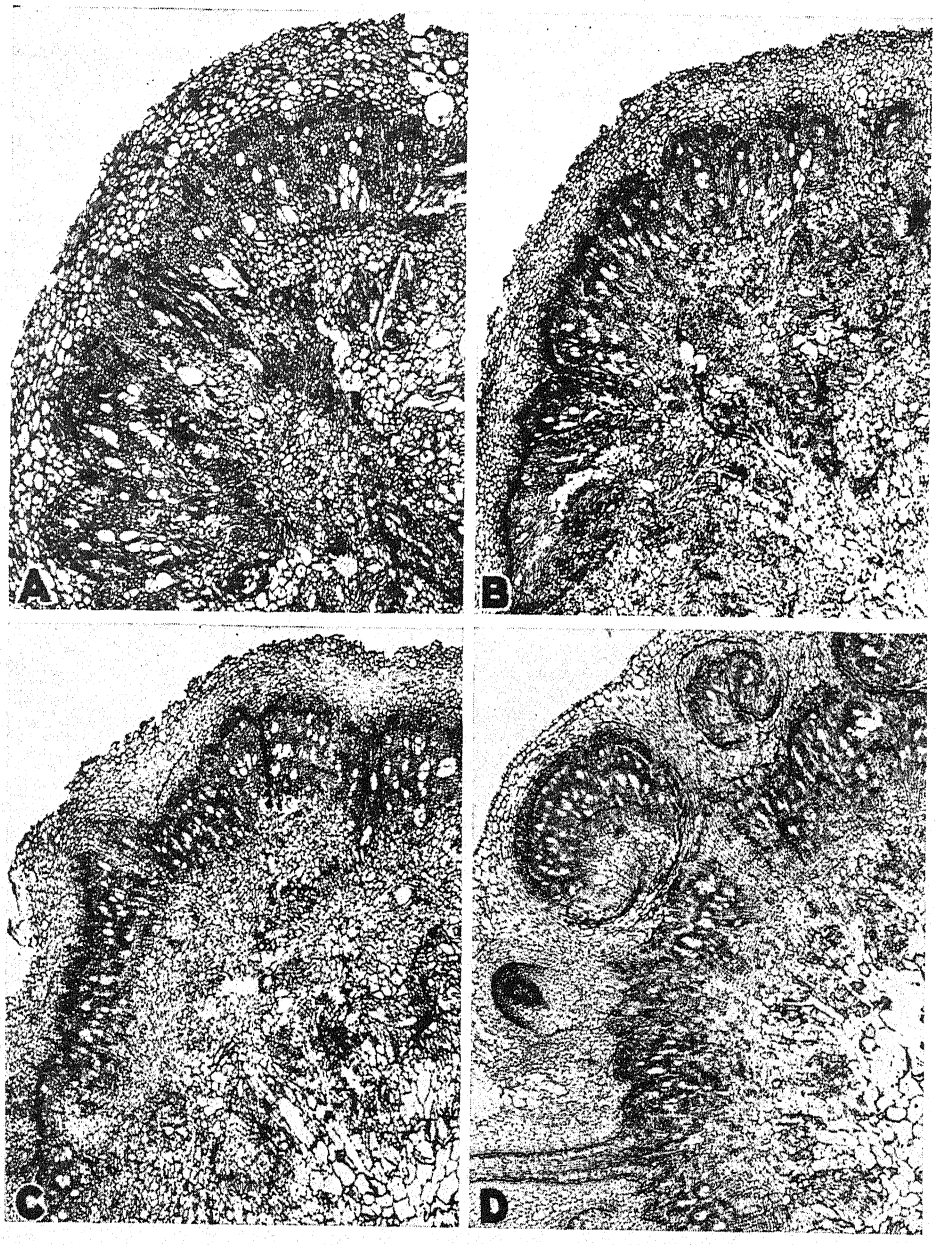


FIG. 17.—Transections of same stem, 18 days after treatment with 0.2% indoleacetic acid. Unusually large and highly vascularized tumor. *A*, 1.5 mm. above cut surface. Periphery meristematic; center highly vascularized with complex of anastomosing strands. *B*, 1 mm. above cut surface. Similar to *A*. *C*, 0.5 mm. above cut surface. Marginal band of vascular tissue, connecting at lower levels with endodermal bundles and vascular cylinder of stele; scattered small strands in center connect with bundles proliferated in pith. *D*, 0.5 mm. below cut surface. Characteristic organization of stem tissues evident; vascular bundles in endodermis, connecting at lower levels with stele; vascular strands from derivatives of pith centrad to protoxylem points.

The results of this study show that at none of the concentrations used did responses to indoleacetic acid duplicate those to tryptophane both in character and in rate of response. A comparison of the histological responses to various concentrations of indoleacetic acid and to tryptophane are presented in table 1. The important differences are as follows:

1. The cortical parenchyma is more reactive with tryptophane than with indoleacetic acid. (Compare fig. 6*D* with 6*B*.)

2. The degree of vascularization in the proliferated endodermis is greater with tryptophane. (Compare fig. 14*B* with 12*B*, 14*A*.)

3. The primary phloem shows little response with tryptophane; with indoleacetic acid it becomes not only active but also vascularized. (Compare fig. 14*B* with 14*A*.)

4. The course of vascularization in these outer tissues is characteristically different for the two substances. With tryptophane the endodermal bundles connect laterally through the outer ray with the stelar cambium and the respective secondary vascular tissues. With indoleacetic acid endodermal bundles pass across the pericycle into the primary phloem and at lower levels connect directly inward through a phloem ray with the cambium and the respective secondary stelar tissues. (Compare figs. 5*D*, 8*B*, 12*C* with figs. 9–11, 12*A*, *B*.)

5. With tryptophane the pith is relatively inactive and disintegrates in the center. It participates but little in the formation of the tumor, which consequently may show a depressed center. With indoleacetic acid the marginal pith actively proliferates and becomes vascularized. The central pith persists as a functional tissue and at the cut surface proliferates to form a considerable part of the cone-shaped tumor. (Compare fig. 1 of KRAUS [6] with our fig. 15*A*, *B*.)

These differences follow closely those found by Kraus in his comparison of the responses to the two substances, except that, as we have pointed out, the initiation of root primordia cannot necessarily be considered a critical point of difference between the effects of the two substances. Although there are certain points of similarity, the dissimilarities are sufficiently characteristic to suggest that the pattern of reactions resulting from the application of tryptophane is different from that upon application of indoleacetic acid, whether in high or low concentrations. On the basis of this histological individuality of effect, there appears to be a distinctive response attributable to tryptophane apart from any response which may result from the conversion of tryptophane to indoleacetic acid.

In addition to histological differences between response to indoleacetic acid and to tryptophane, there are also differences in the time at which various reactions occur. The initial reaction in the endodermis was noted within 24 hours for all the substances described in table 1. In the first 3 days the degree of response to tryptophane, especially in the endodermis, resembles that of 0.02% indoleacetic acid (cf. fig. 2*B*, *D*). Maturation as made evident by differentiation of reticulate tracheids in proliferated tissues was noted by the 3d day with concentrations of 0.2% and 0.02% indoleacetic acid (cf. fig. 6*B*, *D*). However, maturation of vascular elements with tryptophane did not commence until the 6th day (fig. 8*A*, *B*). With concentrations of 0.2% and 0.02% indoleacetic acid, most of the proliferated endodermal cells were matured as parenchyma by the 6th day and with 0.002% by the 3d day. Meristematic activity seemed to continue in the endodermis through the 13th day with tryptophane. In other words, the response to tryptophane was just gaining

TABLE 1

HISTOLOGICAL EFFECTS OF TRYPTOPHANE AND VARIOUS CONCENTRATIONS OF INDOLEACETIC ACID ON BEAN

	Tryptophane (2.0%)	Indoleacetic acid			
		3.0%	0.2%	0.02%	0.002%
Response below cut surface:					
Epidermis	Ruptured at upper levels by expanding inner tissues	Ruptured by roots formed at upper levels	Ruptured by roots formed at upper levels	Remains intact	Remains intact
Cortical parenchyma	Active proliferation at uppermost levels	Outer cells much enlarged; inner cells highly meristematic	Outer cells much enlarged; inner cells meristematic	Cells slightly enlarged; a few divisions	No proliferation
Endodermis	Over major bundles broad arcs of proliferating cells; numerous bundles differentiated from derivatives	Broad band of intensely meristematic tissue, remaining active and participating in root formation; below root zone many derivatives differentiated as vascular tissue	Band of meristematic tissue; over rays derivatives active in root formation; over bundles derived cells matured as parenchyma or differentiated as vascular elements	Proliferated band 5–8 cells wide; slight vascularization	Slight proliferation over major bundles; two to five cells wide in places; matured as parenchyma
Pericycle	Slight response at cut surface; frequently crushed at upper levels	Slightly enlarged and becoming parenchymatous at upper levels; occasionally crushed	Similar to response to 3.0%	Similar to response to 3.0%	Little response
Phloem	Little response	Primary phloem parenchyma highly meristematic and similar to endodermis in root zone; at lower levels proliferation followed by vascularization; some proliferation of secondary phloem parenchyma	Primary phloem parenchyma generally meristematic in areas of root formation; otherwise moderately proliferated; well-organized vascular bundles at lower levels; little response in secondary phloem	Slight proliferation of primary phloem parenchyma; some vascular bundles at lower levels; otherwise inactive	Little response
Cambium	Increased activity	Rapidly increased activity	Increased activity	Increased activity	Slightly increased activity
Xylem	Wide band of compactly arranged secondary tracheids and vessels; medullary ray cells likewise differentiated as tracheids	Formation of moderately wide band of secondary tracheids and vessels with interspersed xylem parenchyma; parenchymatous medullary rays conspicuous	Less wide band of secondary tracheids with interspersed xylem parenchyma	Narrow band of secondary tracheids	A few secondary tracheids differentiated

TABLE 1—*Continued*

	Tryptophane (2.0%)	Indoleacetic acid			
		3.0%	0.2%	0.02%	0.002%
Outer ray	Active; derivatives typically form cambiums connecting vascular structures of endodermis and stele	Meristematic activity similar to primary phloem; forming roots at upper levels; maturing as parenchymatous cells at lower levels	Meristematic; participating in root formation or maturing as parenchyma	Slight activity associated with formation of interfascicular cambium	Inactive; interfascicular cambium infrequent
Inner ray	Inactive, except where derivatives of interfascicular cambium mature as tracheids	Highly active at upper levels, differentiating tracheids, clusters of sieve tubes, and vascular strands	Marked proliferation and differentiation of tracheids and vascular strands at upper levels; inactive at lower levels	Little activity	Inactive
Pith	Slight proliferation of parenchyma opposite xylem points; disintegration of central pith forms cavity	At upper levels activity in pith opposite xylem similar to that of inner ray; central pith remains active	Similar but less in degree than at 3.0%	Slight proliferation at upper levels	No proliferation but generally no disintegration of tissue
Root primordia	None	Numerous	Occurrence variable	None	None
Vascular pattern in outer tissues	Marked vascularization of endodermis with connections to stele laterally through rays	Organization and course of vascular bundles greatly confused by root formation; at lower levels large bundles occurring in endodermis and in primary phloem	Small vascular bundles; at upper levels mainly in endodermis; at lower levels some bundles end blindly, others cross pericycle into phloem and connect directly with vascular tissues of stele	Less well-organized vascular bundles, but following similar course as with 0.2%	None
Development at and above cut surface	At cut surface general proliferation of tissues except central pith; large tumor formed from derivatives of phloem, cambium, and xylem with periphery from endodermis; mainly parenchymatous with small vascular bundles	General proliferation of parenchymatous tissues at cut surface; very large tumor derived mainly from proliferation of pith and becoming highly vascularized	Similar proliferation of parenchymatous tissue at cut surface; very large tumor, derived mainly from proliferation of pith, but outer portions from proliferated xylem, cambium, and phloem; becomes highly vascularized	General proliferation at cut surface; tumor similar in derivation but smaller and less vascularized	General upward proliferation of tissues from phloem inward, forming small parenchymatous tumor

momentum at the time there was a slowing-down in the reaction to 0.02% indoleacetic acid, the concentration most nearly resembling tryptophane. Whether tryptophane is slow in inducing response or whether this retardation is related to a period of time necessary for the conversion of tryptophane to some unidentified growth-regulating substance remain problems.

Summary

1. The histological responses of decapitated bean stems to five low concentrations of indoleacetic acid were studied. Of these concentrations, the two highest—0.2 and 0.02%—induced marked histological changes in the stem and resulted in the formation of a distinct tumor above the cut surface. The intermediate concentration—0.002%—resulted in only slight histological response. The two lowest—0.0002 and 0.00002%—were apparently below the threshold of effectiveness for indoleacetic acid under conditions of this experiment. These last concentrations appeared to induce no greater response than that which occurs in control material. The essential features of the histological responses are summarized in table 1.

2. The responses shown for the three effective concentrations, as well as those described by KRAUS, BROWN, and HAMNER (7) for 3% indoleacetic acid, are essentially of the same pattern. In a stem tip treated with a high concentration, the responses at successively lower levels are similar to the responses which may be induced by treatment with lower concentrations.

3. A comparison of the patterns of response induced by indoleacetic acid and by tryptophane shows many dissimilarities. Significant differences have been described in the type of reaction, the degree of activity, and the vascularization of cortical parenchyma, endodermis, and primary phloem. The character and the degree of response in the pith and its relationship to tumor formation are likewise dissimilar. A more detailed comparison is presented in table 1.

4. At none of the concentrations used did both the character and the rate of response to indoleacetic acid duplicate in entirety the response to tryptophane. On the basis of this histological individuality of effect, there appears to be a distinctive response attributable to tryptophane apart from any response which may result from the conversion of tryptophane to indoleacetic acid.

Data and photomicrographs (figs. 2*D*, 5*D*, 6*D*, 8, 12*C*, and 14*B*) on 3% indoleacetic acid and 2% tryptophane are taken from other papers (5, 6, 7).

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. AVERY, G. S., and BERGER, J. Tryptophane and phytohormone precursors. Science 98:513–515. 1943.
2. BAUSOR, S. C. Effects of growth substances on reserve starch. BOT. GAZ. 104:115–121. 1942.
3. ———. Interrelation of organic materials in the growth substance response. BOT. GAZ. 103:710–724. 1942.
4. BEAL, J. M. Effect of indoleacetic acid on thin sections and detached segments of the second internode of bean. BOT. GAZ. 102:366–372. 1940.
5. HAMNER, K. C., and KRAUS, E. J. Histological reactions of bean plants to growth-promoting substances. BOT. GAZ. 98:735–807. 1937.
6. KRAUS, E. J. Histological reactions of bean plants to l-tryptophane. BOT. GAZ. 102:602–622. 1941.
7. KRAUS, E. J.; BROWN, NELLIE A.; and HAMNER, K. C. Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. 98:370–420. 1936.
8. MITCHELL, JOHN W., and BROWN, JAMES W. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. BOT. GAZ. 107: 393–407. 1946.
9. WENT, F. W., and THIMANN, K. V. Phytohormones. New York. 1937.

RESPONSES OF PTERIS LONGIFOLIA TO APPLICATIONS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 575

BERNARD STRICKLER

Introduction

Studies of the effects of 2,4-dichlorophenoxyacetic acid on plants with but one exception (16) have been confined to the responses of various angiosperms. Prior investigations have tended largely toward the refinement of techniques of applying this substance (17, 12) or an extension of its practical uses (2, 11, 13). The selective sensitivity to 2,4-dichlorophenoxyacetic acid of different species of plants, combined with the resultant telemorphic effects (3), has been of distinct advantage in its use as a herbicide.

In the present experiment information on the action of 2,4-dichlorophenoxyacetic acid is extended to the pteridophytes, especially to the gametophytic generation. The responses of the prothallia suggest the possibility of utilizing this compound as a tool of experimental morphology.

Material and methods

Spores were collected from a single leaf of *Pteris longifolia* grown in the greenhouses of the University of Chicago. Germination and subsequent culture of the gametophytes were carried out on new $2\frac{1}{2}$-inch unglazed clay pots which had been washed and then fired in an electric muffle furnace. The pots were then packed with white quartz sand which had previously been washed with distilled water and autoclaved. They were then inverted in small preparation dishes to which various solutions were added. When the entire pot became wetted, spores were sown on the topmost surface by tapping them from a dissecting needle. Enough solution was then added to submerge just the rim of the pot. The solutions were maintained at this level throughout the experiment by the addition of distilled water. The cultures were covered with transparent, untinted, cylindrical battery jars.

All solutions consisted basically of a one-half dilution of ZINZADZÉ's slightly acid formula (18), pH 6.1. Using this diluted nutrient solution, a stock solution containing 100 mg./l. of the ammonium salt of 2,4-dichlorophenoxyacetic acid was prepared. The ammonium salt was prepared by dissolving 2,4-dichlorophenoxyacetic acid in 95% ethyl alcohol, bubbling anhydrous ammonia gas into the above solution, washing the precipitate with absolute alcohol, and then drying. The final concentrations of the salt used in the experimental series were 50, 10, 1, and 0 mg./l.

The gametophytes received 10 hours of illumination daily from six 36-inch, 30-watt Mazda daylight fluorescent tubes set in a reflector (fig. 1). The temperature of the laboratory in which the prothallia were grown ranged approximately from 75° to 85° F.

Sporophytes used in the experiments were of two ages: small, young plants grown in pots, and large, mature plants growing undisturbed on a soil and rock substrate in the greenhouse. Their petioles and terminal pinnae were treated

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

with concentrations of 2,4-dichlorophenoxyacetic acid ranging from 125 to 2000 p.p.m. These were applied either as lanolin mixtures of the acid or as aqueous solutions of the ammonium salt with Duponol C (9) as a spreader.

Gametophyte and sporophyte material prepared for microscopic examination was fixed in formalin-aceto-alcohol. Whole mounts of the prothallia were stained in Harris' haematoxylin, counterstained in orange G, and mounted in balsam. Sectioned material of the prothallia and sporophytes was prepared according to the tertiary butyl alcohol–paraffin method and stained with either the safranin–fast green combination or Flemming's triple stain.

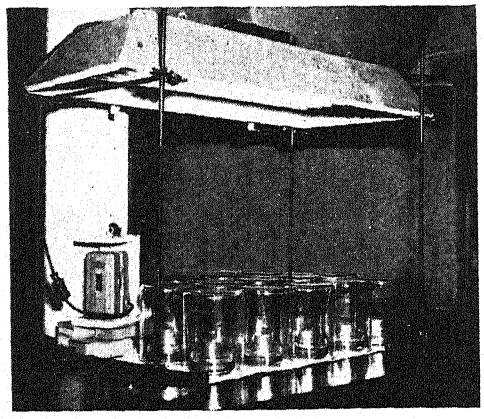


FIG. 1.—Arrangement of cultures of gametophytes under fluorescent lights.

Observations

SPORE GERMINATION

Throughout the course of the studies on the gametophytes, there was a high percentage of germination in all cultures. Nevertheless, in those cultures containing 10 and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate, a larger number of ungerminated spores were conspicuously present than in those containing plain ZINZADZÉ's solution or only 1 mg./l. of the salt. This led to the idea that the presence of this substance at the higher concentrations may have significantly reduced the percentage of germination. The larger size attained by the prothallia in the most dilute and control cultures, however, may have merely obscured the ungerminated spores, so that the visible differences might be more apparent than real.

To test this idea, a series of cultures was set up, according to the methods previously described, in which a number of 5 mm. squares were scratched into the surface of the pots on which the spores were to be planted. In each culture a combined total of 1000 ungerminated and germinated spores was counted. By confining the counts only to spores lying within the squares, the manner of selecting those to be counted was the same for all cultures.

In connection with the standardization of data on the percentage of germination, it should be noted that the significance of such data is affected by the differences in concepts of the definitive stage of spore germination. For example, ORTH (14) designated as germination that stage in development after the splitting of the exospore in which the first rhizoid or some other growth process is evident and chlorophyll is formed. Data based on counts of spores at earlier stages of development would fail in meaning for those who subscribe to ORTH's designation, since the number of plants which attain the requisite stage would be unknown.

Another factor of importance for this type of data is the time interval at which counts are made. DOPP (5) pointed out that the spores of a species of fern, even within the same culture, do not germinate at the same time. The successive

germination of spores of *Pteris longifolia* can be illustrated from our experiment, in which a preliminary count of 600 spores in a control culture revealed that the primary cell had emerged in 244 spores by the seventh day after sowing. Another count of the same area 2 days later showed 419 spores in the same or later stage of development.

For these reasons counts were made 10 days after the emergence of initial prothallial cells was first observed. All counts were made on the same day. Germinated spores then possessed more than one rhizoid and a number of prothallial cells. Ungerminated spores had failed even to split the exospore. The uniformity of germination among all the cultures (table 1) does not support the idea that germination was retarded either in extent or in time by the presence of ammonium 2,4-dichlorophenoxyacetate.

Gametophyte

Growth of the gametophytes was observed from whole mounts of prepared and fresh material collected at 3- or 4-day intervals. All stages of the treated plants were compared with untreated prothallia collected at the same times. Responses of gametophytes to the three concentrations of ammonium 2,4-dichlorophenoxyacetate used—though seemingly quite different at each concentration—showed two types of reactions which were fundamentally the same in all treated cultures: (*a*) an inhibition of growth in size and (*b*) abnormal patterns of development.

Inhibitory effects.—A difference in size was noted in gametophytes which were only 3 or 4 days old (figs. 2, 3). At this stage most plants possessed only one rhizoid; a few had two. At concentrations of 50 and 10 mg./l., the gametophytes were comprised of the single rhizoid and one or two prothallial cells, while those at 1 mg./l. and the control plants had filaments four or more cells long. As already mentioned, spores placed in nutrient solutions containing ammonium 2,4-dichlorophenoxyacetate were not delayed in germination so that this difference cannot be ascribed to age. That such is not the case is obvious from observations made on older gametophytes. Thirteen-day-old gametophytes at 50 mg./l. were still filamentous, consisting of about seven prothallial cells and about six rhizoids (fig. 4). At all other concentrations the gametophytes were thalloid; the smallest being those grown at 10 mg./l.—the largest, the control plants (figs. 5, 6, 7). By the 27th day the untreated gametophytes had formed the typical heart-shaped prothallium; those at 1 mg./l. had begun to form a second lobe; the gametophytes at 10 mg./l. consisted of a single lobe, with no indication of forming a second one, while those at 50 mg./l. had remained essentially filamentous except for a small amorphous mass of cells near the apex.

TABLE 1

Results of tests of germination of spores of Pteris longifolia in ammonium 2,4-dichlorophenoxyacetate

Concentration of salt (mg./l.)	Ungerminated spores	Germinated spores	Total	Percentage of germination
0.....	257	748	1005	74.4
1.....	252	753	1005	74.9
10.....	247	756	1003	75.3
50.....	254	773	1027	75.1

Measurements of gametophytes when they were 3 and 27 days old showed that the size of prothallia grown at 50 mg./l. had increased only 3.39 times; at 10 mg./l., 48; at 1 mg./l., 113; while the control prothallia had increased 180

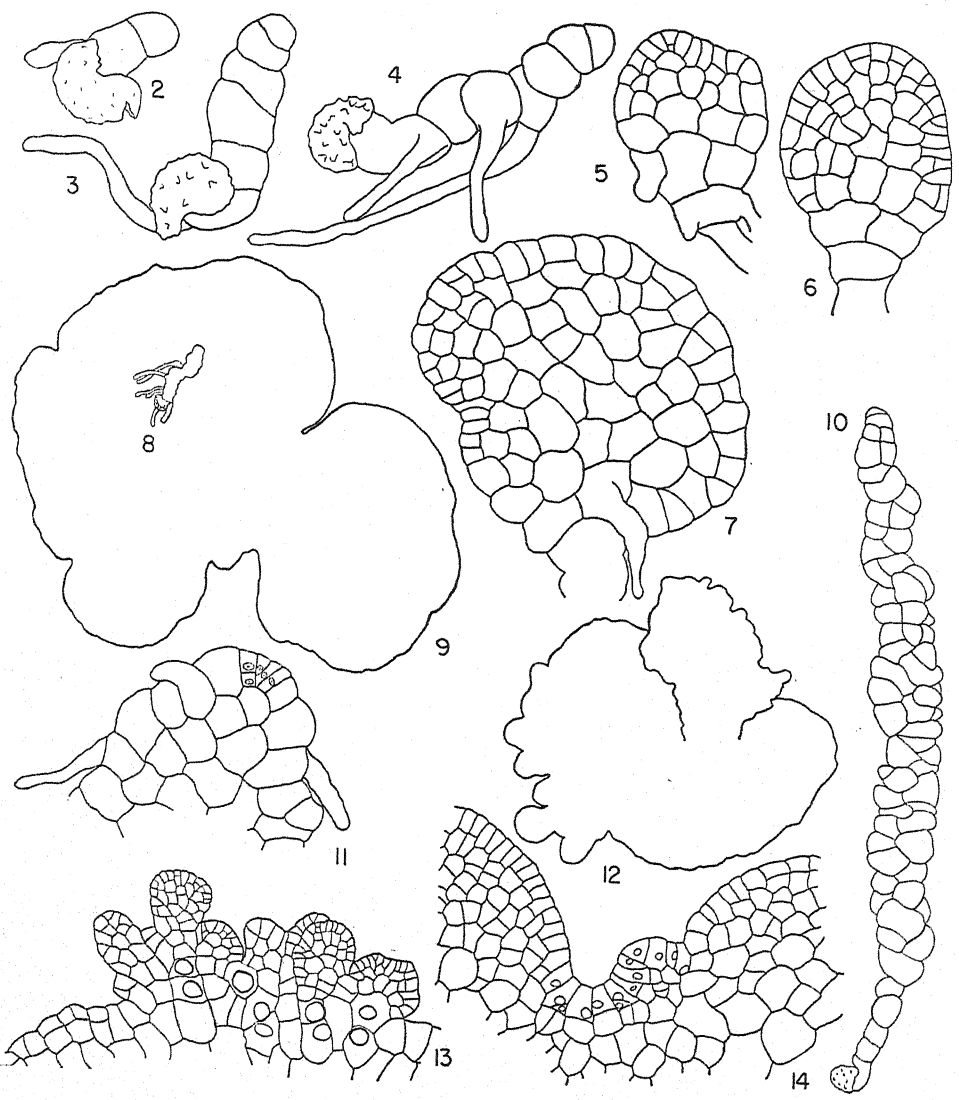


FIG. 2–14.—Gametophytes grown on various concentrations of ammonium 2,4-dichlorophenoxyacetate in ZINZADZÉ's nutrient solution (rhizoids mostly omitted). Figs. 2, 3, gametophytes 3 days old grown at 50 and 0 mg./l., respectively. Figs. 4–7, relative size of gametophytes 13 days old grown at 50, 10, 1, and 0 mg./l., respectively. In fig. 7 the meristem primordium is seen in typical lateral position on first lobe. Figs. 8, 9, relative size of gametophytes 30 days old at 50 and 0 mg./l., respectively. Fig. 10, gametophyte grown for 4 months at 50 mg./l., showing hemispherical end cell and juvenile filamentous condition. Fig. 11, apical portion of prothallium 27 days old, grown at 10 mg./l., with apical position of meristem on single thallus lobe. Fig. 12, prothallium 51 days old, at 1 mg./l., with vertical third wing and secondary prothallia on basal portion of thallus. Fig. 13, details of secondary prothallia of fig. 12. Fig. 14, early stage in development of third wing from base of sinus.

times. These values are based upon measurements of camera lucida drawings of the surface areas of the gametophytes. Each value represents the average of several gametophytes.

ALBAUM (1) has shown that, for fern prothallia composed essentially of a single layer of cells, surface area is a fair index of the mass of tissue present at any time and that increase in surface area may thus be used as an index of growth. Beyond the 27th day the prothallia in the present experiment developed more than a single layer of cells. After this, surface area was not used as an index of growth.

Differences in size between gametophytes of the same age grown at different concentrations of the salt were due chiefly to differences in cell number. The largest cells in the treated prothallia (figs. 4, 5, 6) were equal in size to those of untreated prothallia of the same age (fig. 7). This indicates that the two growth phases—cell division and cell enlargement—were dissimilarly affected by the experimental solutions. This is clearly shown in the prothallium in figure 5. Here the small number of larger, central cells composing the bulk of the thallus are surmounted by a marginal row of smaller cells of similar size, the "Randmeristem" described by ORTH. Intermediate-sized cells (fig. 7), which normally lie between the marginal meristematic cells and the more mature basal and central cells, are for the most part lacking, indicating that cell division but not cell enlargement was inhibited.

Striking evidence of the inhibitory action of the salt is seen in the comparison of 30-day-old gametophytes grown at a concentration of 50 mg./l. with control prothallia of the same age (figs. 8, 9).

FORMATIVE EFFECTS AT 50 MG./L.—There was no evidence of plate formation in gametophytes grown at 50 mg./l. until 30 days after germination. Until this time they consisted of a linear series of cells always derived from divisions of the hemispherical terminal cell. The filament was distorted, owing to the unequal distension and consequent bulging of the wall of each maturing cell. Except for the formation of rhizoids, divisions of these derivatives were rarely seen. After the 30th day subsequent derivatives of the end cell divided in an irregular manner. Ultimately, an elongated clump of distended cells was formed, comprising the only "thallus" seen in these gametophytes throughout the experiment. From the small size of the cell mass, attained over a period of 4 months, it is unlikely that the lineage from each derivative of the end cell was more than a single cell (fig. 10).

The appearance of these retarded gametophytes is unlike that commonly described for gametophytes whose juvenile stage has been prolonged by subjection to a variety of other abnormal conditions, particularly those of light (4, 10). These latter filaments are almost always attenuated, branched, and usually produce antheridia. None of these characteristics was seen in the present gametophytes; they formed no sex organs, were never branched, and the cells, as already indicated, were not attenuated.

GAMETOPHYTES GROWN AT 10 MG./L.—These gametophytes showed two interesting types of peculiarities, the first of which concerns the position of the meristem. Although every gradation between the apical and lateral position of the meristem primordium is found among ferns with cordate prothallia (6), the prothallium of *P. longifolia* typically develops from a lateral meristem.

Development of the untreated gametophytes conformed to the usual type of

development of *P. longifolia* as described by GOEBEL (6, p. 204): "The surface of the prothallus which first develops from the germ-thread becomes at once the one wing, and the meristem which forms the vegetative point of the prothallus comes thereby to occupy a lateral position, and underneath it the second wing of the prothallus shoots out. Figure 150 exhibits this process in *Pteris longifolia*.[2] Here it will be seen that a one-layered cell surface is formed first of all from the germ-thread without the aid of an apical cell, and the anticlinal walls diverge at the apex. . . . . The intensity of the cell multiplication remains strongest at a lateral position on this cell surface, and there is the meristem in which often a two-sided apical cell is visible." In all the untreated plants observed in the present study, the meristem always arose laterally on the first formed wing (fig. 7).

Gametophytes treated with 10 mg./l., on the other hand, consistently formed their meristem apically. In this respect, these prothallia conformed to similar stages described for *Pteridium aquilinum* (14) and *Onoclea struthiopteris* (5, 14), in which the meristem typically lies in the longitudinal axis of the prothallium (fig. 11).

Because of the slow rate of cell division in treated gametophytes, the segments formed on either side of the meristem failed to extend beyond the vegetative point; thus, these prothallia did not become heart-shaped (fig. 15).

The second peculiarity was observed in prothallia 51 days old, on which a thick cushion consisting of distended, loosely arranged cells covered most of the ventral surface. In nearly all prothallia a prominent, compact tubercle was growing within the cushion close to the apex. A few prothallia had two tubercles lying side by side in the same forward position. In other prothallia these masses projected above the dorsal surface (fig. 15), but for the most part they were confined to the ventral surface.

The tubercles consisted of a large number of small, densely cytoplasmic cells with large nuclei (fig. 16). The cells did not exhibit any correlation but appeared to have divided at random. The entire cell mass was inclosed by the large vacuolate cells of the cushion.

Nearly all prothallia observed were without sex organs. Rarely, one or two unopened archegonia were seen on the cushion.

GAMETOPHYTES GROWN AT 1 MG./L.—Increase in size at this concentration also proceeded at a slower rate than in plain ZINZADZÉ's solution so that at the time of sex-organ formation these prothallia were considerably smaller than the control plants. Up to the 30th day, the gametophytes followed the usual pattern of development and attained the cordate shape. Subsequently, the cushion in the treated plants became more massive and extensive than that formed by the controls. Sex organs which later appeared on the cushion were more numerous on treated plants; especially antheridia.

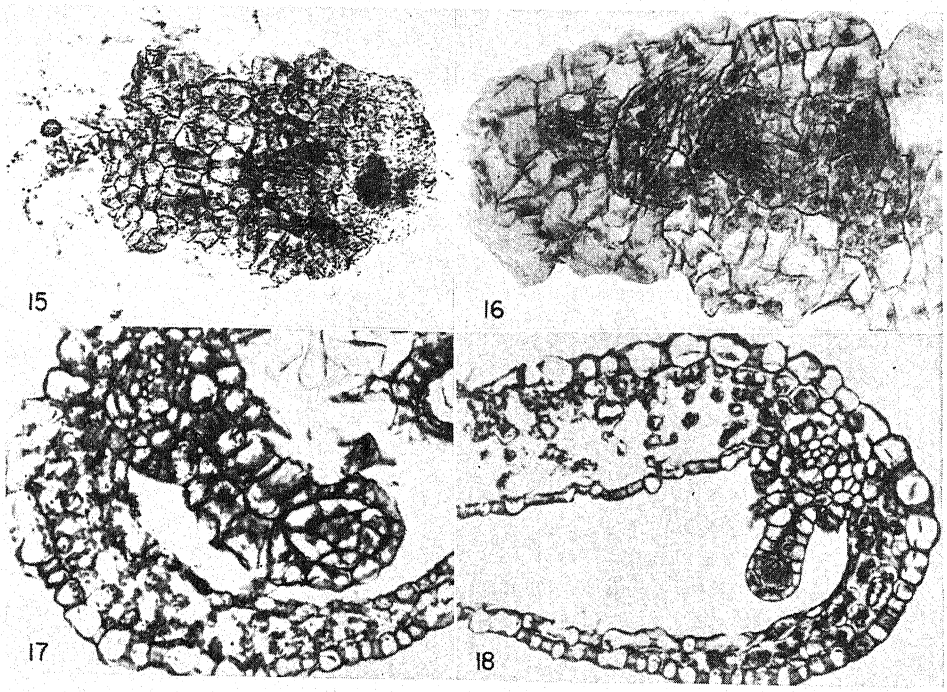
In plants treated at the 1 mg./l. concentration of ammonium 2,4-dichlorophenoxyacetate, there followed next renewed vegetative activity of the apical meristem, first evidenced by a small, tongue-like projection lying close to the base of the sinus formed by the two wings (fig. 14). Elongation of the projection proceeded in an upward direction accompanied by rapid lateral expansion. Thus, in 51-day-old gametophytes the meristem was borne at the apex of a third wing, usually fan-shaped with undulating margins (fig. 12), but some-

[2] Figure 7 of the present paper shows an equivalent stage to fig. 150 of Goebel.

times with margins curled to form a cylinder.

The vertical position of this wing permitted illumination on both sides so that it was not surprising to find sex organs on both surfaces. In addition, archegonia were present on the margin of the apex on either side of the meristem.

process were observed, but, by tracing cell derivatives, it is clear that marginal cells became meristematic, forming a plate directly, with no intervening filamentous stage (fig. 13). Owing to the simultaneous activity of many marginal cells, the adventitious thalli were crowded.



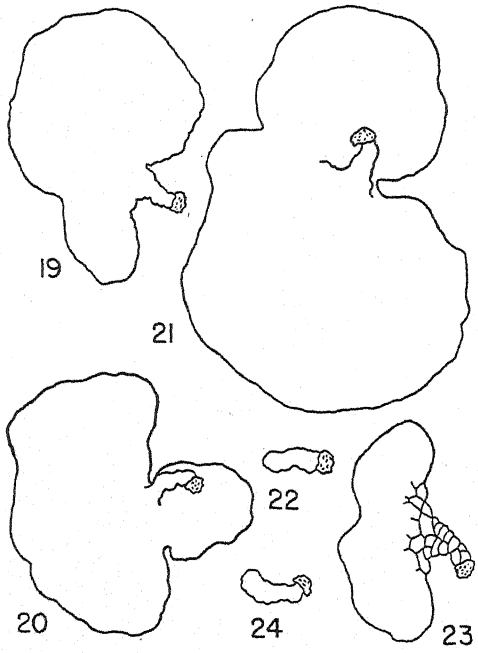
FIGS. 15–18.—Fig. 15, prothallium grown at 10 mg./l. solution of ammonium 2,4-dichlorophenoxyacetate, showing tubercle in apical region. Fig. 16, longisection of tubercle showing random arrangement of small, densely cytoplasmic cells. Fig. 17, transverse section of pinna treated with lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid, showing grossly enlarged sporangium and receptacle. Fig. 18, pinna of control.

Simultaneously with the formation of the third wing in 51-day-old gametophytes, secondary prothallia were growing from the basal portion of the old wings (figs. 12, 13). Each of these new growths was intimately attached to the old gametophyte by means of a broad base, cells of which evidently were part of the parent tissue. Many of these older cells bore antheridia. No initial stages of this regeneration

Sex organs on plants treated with 1 mg./l. of ammonium 2,4-dichlorophenoxyacetate were functional, young sporophytes appearing on most prothallia in 120-day-old cultures. No irregularities were observed on primary leaves, and such plants were transplanted to agar in Petri dishes where they grew well for 4 months and gave every indication of continuing vigorous growth. In all respects these young sporophytes were

comparable to those formed at the same time on control prothallia.

TRANSPLANT EXPERIMENT.—Figures 19–24 show the results of experiments made to determine (*a*) the effect of 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate on young prothallia not previously treated and (*b*) the permanency of the effect on gametophytes germinated and grown for a period of time on this same substance. The methods were the same as those previously described, except that the spores were sown on squares of glass cloth placed on the upper surface of inverted pots instead of directly on the pot surface. In this way prothallia were easily transferred from one pot to the other.



FIGS. 19–24.—Fig. 19, prothallium 21 days old grown from spore on plain nutrient solution. Fig. 20, prothallium grown for 21 days on plain nutrient solution, then transferred and grown for 12 days on 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Fig. 21, prothallium grown continuously on plain nutrient solution for 33 days. Figs. 22–24, reciprocal of experiment shown in figs. 19–21.

Figure 19 shows a prothallium which was grown for 21 days on plain ZINZADZÉ's solution. A number of prothallia of this age were then transferred to the cultures containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. After 12 days these gametophytes (fig. 20) were compared with those of the same age—now 33 days old—which had not been transferred (fig. 21).

It is readily seen that the retardation of cell division is not a response in which the initial treatment of the germinating spore is a necessary condition but that the inhibitory action of ammonium 2,4-dichlorophenoxyacetate is similarly effective on later stages of prothallial development.

In the reciprocal experiment, spores were germinated and grown on cultures containing 50 mg./l. of the salt. After 21 days the minute filaments (fig. 22) were transferred to plain ZINZADZÉ's solution. Within 3 or 4 days the apex of the gametophytes had broadened considerably, and at the end of 12 days the two wings were well established (fig. 23). During this 12-day period the gametophytes which had remained in contact with the nutrient solution containing the growth-regulating substance showed no appreciable increase in size (fig. 24).

Renewed meristematic activity of transplanted prothallia was also observed in the tuberculate plants resulting from the 10 mg./l. treatment. A number of these gametophytes were transplanted to agar plates in an effort to induce further growth of the tubercles. Instead, germinal filaments soon emerged from marginal and inner cells of the thallus. These filaments formed the usual spatulate lobe, developed a meristem, and became cordate. The original prothallia died.

It is apparent from the regenerative growth of the transplanted prothallia

that the inhibitory activity of ammonium 2,4-dichlorophenoxyacetate does not persist once the plants are removed from actual contact with the effective solution. This behavior is not wholly unexpected when one considers that each cell of the thallus is potentially capable of reproducing a new prothallium and that the amount of the substance in a single cell at any one time must be exceedingly small. The derivatives of such a cell, having once succeeded in dividing, would then rapidly become removed from the influence of the residual substance.

Sporophyte

Applications of lanolin mixtures and aqueous solutions were made at the basal portion of the petioles, usually below the first pinna, at the time the leaves had unfolded about one-half their ultimate length. The mature plants at this time were producing leaves 40 cm. or more in length, while the leaves of the young potted plants were rarely 15 cm. long. Applications on the mature plants were at 500 and 1000 p.p.m.; the young plants, in addition to these concentrations, were treated with 125, 250, and 2000 p.p.m.

Gross reactions.—Within 24–48 hours after treatment the petioles at various distances from the point of application showed pronounced downward bending, except at a concentration of 125 p.p.m. at which there was no reaction. As the croziers slowly unrolled, the petioles continued bending in one direction or another until the full length of the leaf was attained. In all cases the final length was less than that of the controls.

Reactions of the pinnae were primarily characterized by a failure to unfold completely (fig. 25*d*). For a period of 3 or 4 weeks after treatment the pinnae slowly continued to unroll and expand, after which they persisted in one or another of the stages of unfolding. Retardation in this respect was greatest in the uppermost (i.e., the youngest) pinnae and decreased basipetally to the pinnae which were fully expanded at the time of treatment. These latter pinnae exhibited no effects from the treatments.

Intensity of responses appeared to be related more to the relative age of leaves at the time of application than to concentrations used. In preliminary experiments numerous applications were made on leaves of similar ages, and the intensity of responses showed considerable variation in which the most marked reactions were not always associated with the highest concentrations used (figs. 25*a*, *b*, *c*). Responses were more rapid to aqueous solutions than to the lanolin mixtures.

Histological reactions.—Several days after the curvatures of the petioles appeared, the leaves became unusually inflexible, the young pinnae and young portions of the petioles acquiring a brittle quality. Although mature petioles of *P. longifolia* possess a natural toughness because of the thick-walled epidermis and the several layers of thickened outer cortical cells, newly differentiated parts of the petiole are supple.

Transverse sections of these young parts showed that the subepidermal layers of the cortex were already considerably thickened, hence more mature than comparable tissues of the control plants. Tertiary thickening of the metaxylem elements also occurred earlier in the treated plants, but this was evident only in a small number of elements surrounding the invaginated phloem in the arms of the horseshoe-shaped stele. The most pronounced thickening of the vascular elements occurred in the treated basal portions of petioles of mature

plants; especially where the aqueous solution of ammonium 2,4-dichlorophenoxyacetate was used.

Aside from the early maturation of these tissues, and a slightly greater thickening of all the cortical and stelar tissues, no other histological irregularities of the petioles were evident. There were no secondary outgrowths, tumors, proliferations, or derangements in the position of the tissues.

THE SPOROPHYLL.—The terminal pinnae, from which the observations of the reactions of the sporangium were made, were treated when the pinnae were completely unfolded and the indusium was still closed. By the use of a hypodermic syringe, a thin, uniform thread of the lanolin mixture of the acid (500 p.p.m.) was laid along the indusium on one side of the pinna. The indusium on the other side received no treatment. On a second pinna a thin band of the aqueous solution of ammonium 2,4-dichlorophenoxyacetate (500 p.p.m.) was painted on one indusium with a camel's-hair brush. These treatments were repeated on several plants.

Within 2 days after application there was a marked epinasty of the treated

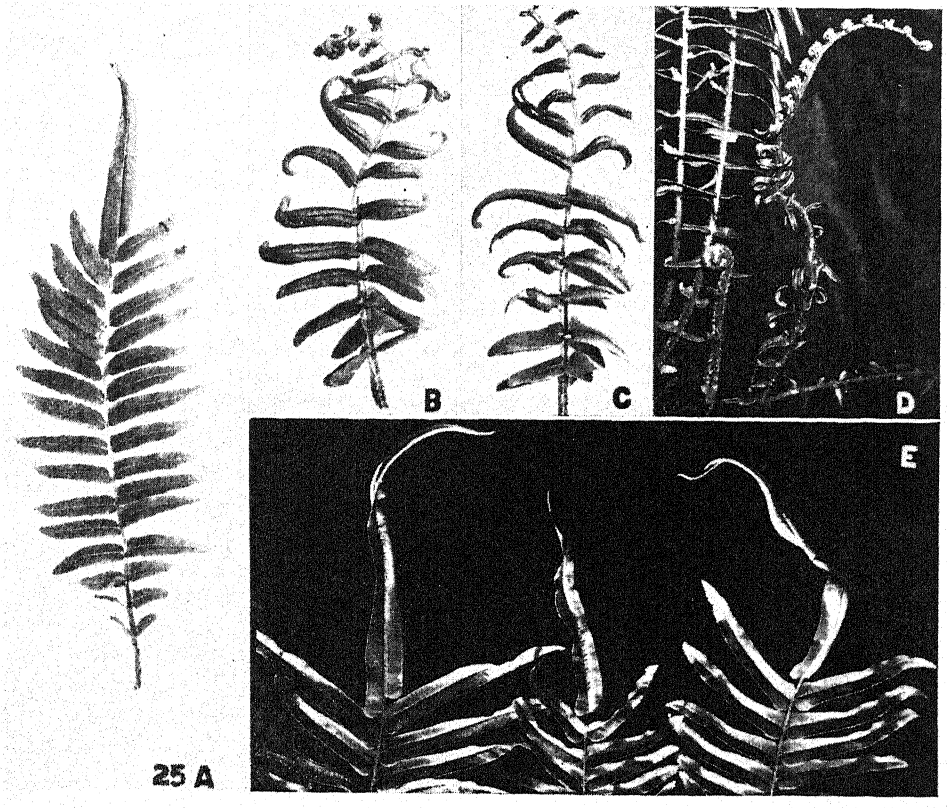


FIG. 25.—Fig. 25*A–D*, leaves 30 days after treatment with ammonium 2,4-dichlorophenoxyacetate: *A*, control; *B*, 500 p.p.m.; *C*, 2000 p.p.m.; *D*, 1000 p.p.m., leaf of mature plant showing marked failure of pinnae to unroll. Fig. 25*E*, terminal pinnae 14 days after unilateral treatment: *left*, lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid; *middle*, lanolin control; *right*, aqueous solution of 500 p.p.m. ammonium 2,4-dichlorophenoxyacetate.

halves of the pinnae. By the 4th day the pinnae treated with the aqueous solution showed a spiral twisting along the axis of the treated indusium. Similar but less pronounced responses followed the application of the lanolin mixture. In preliminary experiments pinnae so treated persisted in the twisted condition for an indefinite time without further gross responses. The present material, however, was collected at the end of 14 days (fig. 25*e*).

Histologically, the pinnae were little affected by the growth-regulating substance. Transections showed enlargement of cells of the upper epidermis associated with the curvatures on the treated side of the blade, and there was also some derangement of the palisade layer accompanying this same response.

In contrast to the inactivity of the pinna in general, the quantitative reaction of the sporangia and receptacle was very striking. It was characterized by pronounced cell enlargement which resulted in the growth of these structures to more than twice their usual size.

The receptacle of *P. longifolia* is not prominent, being only slightly elevated above the plane of the lower epidermis. The receptacle beneath the treated indusium, however, became a rather massive structure which protruded well beyond the epidermis (fig. 17). The receptacle on the untreated side of the pinna was relatively unaffected (fig. 18).

A comparison of figures 17 and 18 also shows that the sporangia beneath the treated indusium were markedly affected and that the cells of these sporangia, particularly those of the stalk and tapetum, exhibited the same swollen condition as the receptacular cells. Although the sporangia and receptacles are meristematic up to the time the indusium is fully open and the sporangia are mature, these organs were not stimulated to proliferate. The number of cells comprising the receptacle, and especially the sporangia, were always consistent with the particular stage of development in which they were observed.

Discussion

The results of the experiments on the gametophytes show that the primary and general effect of ammonium 2,4-dichlorophenoxyacetate was inhibition of growth. This effect was essentially the result of the retardation of cell division with little or no effect upon cell enlargement. In contrast to the behavior of the sporophytes, the quantitative responses of the prothallia were directly correlated with the concentration of the effective substance. Thus, at 50 mg./l. retardation was so great that no plate was formed, whereas at 1 mg./l. the gametophyte developed into a heart-shaped prothallium with functional sex organs.

Aside from the obvious inhibitory effect on growth of the gametophytes, it is less apparent whether the unusual structures observed on the prothallia were the result of the direct influence of ammonium 2,4-dichlorophenoxyacetate or whether these growths were secondary responses resulting from other causes.

For example, it is not certain whether the tubercles which appeared on prothallia grown at 10 mg./l. were apogamous embryos whose differentiation was unorganized due to the influence of the growth-regulating substance or whether they were gall-like masses resulting from proliferation of the cushion cells.

HEIM (8) has described certain projections from the cushion of *Doodya caudata* from which apogamous sporophytes arose, and the internal appearance of these structures as figured by him bear a resemblance to that of the present tuber-

cles. In the case of *Doodya*, however, the sporophytic nature of the projections was confirmed at a relatively early stage by the development of tracheids, later followed by the formation of the apices of the leaf, stem, and root of the embryo, which then grew in a normal manner.

Usual evidences of apogamy in ferns (15), such as the formation of tracheids, or hairs surrounding the embryo, were not present in the treated prothallia, and the cell masses as seen in longitudinal and transverse sections were not recognizable embryos. In addition, transplants made to induce further growth of the tubercles failed. It does not appear likely that the tubercles were sporophytic.

The renewed activity of the apex of prothallia grown at 1 mg./l. also suggests the possibility of secondary formative influences. It is well known that the gametophytes of the leptosporangiate ferns are much more plastic than the sporophytes and that relatively minor alterations of the environment of the prothallia induce profound form changes.

In this connection it is interesting that the outgrowth of the apex in the treated prothallia is strikingly similar to that obtained by ALBAUM (1) in young prothallia which were changed from their nearly vertical position to a horizontal one. Following the reorientation, the apex in these prothallia began to grow upward toward the source of light, and there was ultimately formed a third wing similar to that shown in figure 12. It is not to be inferred, however, that the apical outgrowths in the treated prothallia necessarily originated in this same manner, since their position relative to the source of light was not experimentally altered.

The curvatures of petioles and pinnae following treatment of the leaves of *P. longifolia* indicate that the reactions of the sporophyte of this fern to 2,4-dichlorophenoxyacetic acid are not markedly different from those of various angiosperms that have been similarly treated.

In the first report of the activity of this substance on plants, ZIMMERMAN and HITCHCOCK (17) designated as a general type of response "the negative curvatures of stems and leaves, i.e., responses involving cell elongation." Subsequent studies by these investigators and others have shown that distorted growth of stems and leaves is readily induced in many angiosperms by applications of 2,4-dichlorophenoxyacetic acid and is one of the first conspicuous effects of such treatments. In the same paper, ZIMMERMAN and HITCHCOCK described a second type of effect—"morphogenetic activity." This term was used to refer to "modifications of organs which involved responses other than curvatures due to cell elongation." Except for the modification of sporangia, which was basically the result of cell enlargement, this second type of response was strikingly absent in the treated sporophytes. The tissues of the leaves were relatively unaffected by the applications of 2,4-dichlorophenoxyacetic acid or its ammonium salt and were quite insensitive to the formative influences of this substance. The resistance of the sporophyte of *P. longifolia* to histological modification when treated with 2,4-dichlorophenoxyacetic acid is not unique but serves as another example emphasizing the selective action of this substance.

Summary

1. Spores of *Pteris longifolia* were germinated and the gametophytes were grown uninterruptedly with ZINZADZÉ's

solution containing 0, 1, 10, and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate.

2. Spore germination was not adversely affected either in time or in percentage by the presence of the growth-regulating substance.

3. The smaller size of treated gametophytes was due to the smaller number of cells comprising them and not to the size of the cells. The intensity of retardation of cell division was proportional to the concentration of the ammonium salt used.

4. Treatment at 10 mg./l. resulted in the formation of the meristem primordium in the apical instead of the usual lateral position. These prothallia did not become heart-shaped and rarely produced sex organs.

5. Fifty-one-day-old gametophytes growing at 10 mg./l. exhibited tubercles consisting of small, densely cytoplasmic cells in random division within the massive cushions.

6. Prothallia growing at 1 mg./l. showed renewed vegetative activity of the meristem to form a third wing. Simultaneously, secondary prothallia were growing from the basal portion of the old prothallial wings. Functional sex organs were more abundant on the prothallia grown at this concentration than on the controls.

7. Young prothallia grown for 21 days on plain ZINZADZÉ's solution made no appreciable growth after being transferred to a culture containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Conversely, gametophytes which were germinated and grown for 21 days on the latter culture showed a rapid recovery to normal growth after being transferred to plain ZINZADZÉ's solution. Tuberculate prothallia transferred from 10 mg./l. cultures to agar plates quickly produced secondary prothallia which were normal in all respects. The tubercles made no further growth.

8. Leaves of young and old sporophytes of *P. longifolia* were treated with lanolin mixtures of 2,4-dichlorophenoxyacetic acid (500 p.p.m.) and aqueous solutions of the ammonium salt of the acid (125–2000 p.p.m.). The petioles showed marked curvatures and the unrolling of pinnae was retarded. These responses were permanent. There was no reaction from applications of 125 p.p.m.

9. Histologically, the applications resulted in earlier maturation of the petioles, especially in the outer cortex and the metaxylem elements surrounding the invaginated phloem. There were no tumors, proliferations, or derangement of the tissues. The responses were more marked where the aqueous solutions were used.

10. Unilateral treatment (500 p.p.m.) of terminal pinnae resulted in epinasty and spiral twisting of these organs. Beneath the treated indusium, the receptacle and sporangia were grossly enlarged due to cellular distension. There was no unusual increase in the number of cells comprising these structures.

The writer acknowledges with sincere thanks the advice and aid given by P. D. VOTH during the course of this investigation.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. ALBAUM, H. G. Normal growth, regeneration, and adventitious outgrowth formation in fern prothallia. Amer. Jour. Bot. 25:37–44. 1938.
2. BATJER, L. P., and MARTH, P. C. New materials for delaying fruit abscission of apples. Sci. n.s. 101:363–364. 1945.
3. BEAL, J. M. Further observations on the telemorphic effects of certain growth-regulating substances. BOT. GAZ. 106:165–178. 1944.
4. BOWER, F. O. The ferns. Cambridge University Press, London. 1926.
5. DOPP, W. Untersuchungen über die Entwicklung von Prothallien einheimischer Polypodiaceen. Pflanzenforschung 8. Gustav Fischer, Jena. 1927.
6. GOEBEL, K. Organography of plants. Clarendon Press, Oxford. 1905.
7. HAMNER, C. L., and TUKEY, H. B. The herbicidal action of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on bindweed. Sci. n.s. 100:154–155. 1944.
8. HEIM, C. Untersuchungen über Farnprothallien. Flora 82:329–373. 1896.
9. Industrial and Engineering Chemistry 35:127. 1943.
10. KLEBS, G. Zur Entwicklungs-physiologie der Farnprothallien. Sitz. d. Heidel. Akad. Wiss. Math-Nat. Klasse. Pp. 1–138. 1917.
11. MARTH, P. C., and MITCHELL, J. W. 2,4-Dichlorophenoxyacetic acid as a differential herbicide. BOT. GAZ. 106:224–232. 1944.
12. MITCHELL, J. W., and HAMNER, C. L. Polyethylene glycols as carriers for growth-regulating substances. BOT. GAZ. 105:474–483. 1944.
13. MITCHELL, J. W., and MARTH, P. C. Effects of 2,4-dichlorophenoxyacetic acid on the ripening of detached fruit. BOT. GAZ. 106:199–207. 1944.
14. ORTH, R. Morphologische und physiologische Untersuchungen an Farnprothallien. Planta 25:104–149. 1936.
15. STEIL, W. N. Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. 5:433–453. 1939.
16. STEVENSON, E. C., and MITCHELL, J. W. Bacteriostatic and bactericidal properties of 2,4-dichlorophenoxyacetic acid. Sci. n.s. 101:642–644. 1945.
17. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–344. 1942.
18. ZINZADZÉ, C. Nutrition artificielle des plantes cultivées. Annales agronomiques. n.s. 2:809–853. 1932.

THE EFFECT OF INDOLEACETIC ACID UPON INITIATION AND DEVELOPMENT OF HYPOCOTYLEDONARY BUD PRIMORDIA IN FLAX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 576

GEORGE K. K. LINK AND VIRGINIA EGGERS[1]

Introduction

Generally, the normal nondecapitated hypocotyl of *Linum usitatissimum* develops neither buds nor a shoot. If it does, the buds and shoot are restricted to the base of the hypocotyl (2, 6). If decapitated at a suitable time, however, the hypocotyl generally develops buds abundantly at all levels and, in time, a shoot (generally, one only completes development) which substitutes for the lost axis (2). In the second paper of this series, LINK and EGGERS (6) reported that, while bud primordia are initiated in the lower half of the intact hypocotyl of flax, none are initiated in its upper half. Following decapitation, however, the upper half, too, initiates bud primordia, and the lower half probably initiates some in addition to those present before decapitation.

[1] This is the third of a series of papers reporting the results of studies of hypocotyledonary and epicotyledonary vegetative buds and wound tissues of *Linum usitatissimum* by GEORGE K. K. LINK and VIRGINIA EGGERS which have extended from 1934 to date. The work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

The study reported here was undertaken on the assumption that the cotyledons and epicotyl have an inhibitory effect on the initiation and development of hypocotyledonary bud primordia in flax and that this effect is brought about by an auxin or by a complex involving one or more auxins in proper concentration. The decapitated hypocotyl of flax, accordingly, was used to test the effect of indoleacetic acid in lanolin upon the formation and development of hypocotyledonary buds when applied to the decapitation cut. The primordium of a hypocotyledonary bud of flax is initiated by transverse division of an epidermal cell without special prior enlargement or increase in protoplasm of the cell about to divide (5, 6). Therefore, it seemed that this study might test the effect of indoleacetic acid upon division of a cell—without prior enlargement of the cell—as manifested in the initiation and in the early stages of development of a hypocotyledonary bud primordium in the upper half of the hypocotyl, as well as upon the complex of cell enlargement, cell division, and cell differentiation manifested in subsequent development and growth of the young bud into a shoot.

Material and methods

Seed of the Bison strain of *L. usitatissimum* was planted in quartz sand in 6-inch pots watered with the complete mineral nutrient solution used by EGGERS (3). The plants were grown in the greenhouse, and plantings were made throughout the year. Seedlings were thinned so as to leave 20–25 plants per pot. Some plants were left as uncut controls, but most were decapitated directly below the cotyledons, generally on the 10th day after emergence of the seedlings from the substrate. The necessary number of decapitated plants was used as untreated cut controls, and the others were used for treatment with lanolin alone or with indoleacetic acid in lanolin in various concentrations. The concentrations tested ranged from 3% indoleacetic acid to 0.0000012% (4.5×10^{-7} molal). In preparation of concentration 0.116% indoleacetic acid (6×10^{-3} molal and designated 1:1 in this paper), one part of saturated aqueous solution of indoleacetic acid was stirred into an equal part of anhydrous lanolin. From this standard mixture higher dilutions were prepared by addition of proper amounts of lanolin. The lanolin alone and the lanolin–indoleacetic acid mixtures were applied to the cut surfaces of the hypocotyls in sufficient quantity to cap the stumps. In some experiments only one application was made; in others they were made daily.

In experiments 1–5, one pot each with 20–25 plants was used as a test lot, and the plants were observed daily. Readings were made at definite intervals in terms of the number of plants budded and of the number of buds per budded plant. Results of the former readings are expressed as percentages of plants budded per test lot; results of the latter, as the average number of buds per plant of each test lot.

Variability of individual plants as to bud formation and development was found to be so great that data were required from which the standard error (SE) and the value of t could be calculated. To this end experiments 6 and 7 were set up.[2] In experiment 6 each test lot consisted of five pots, each with twenty plants, and in experiment 7 each test lot consisted of six pots, each with

[2] We are indebted to our colleague Professor SEWALL WRIGHT for counsel and advice in setting up these tests and for the equations used to determine SE and t.

twenty plants. The value of SE was calculated according to the equation

$$SE = \sqrt{\frac{(\Sigma V_1^2 - \bar{V}_1 \Sigma V_1) + (\Sigma V_2^2 - \bar{V}_2 \Sigma V_2)\left(\frac{1}{n} + \frac{1}{n}\right)}{2n - n}},$$

in which V is the average number of buds per plant in each pot of each test lot; n is the number of pots in each test lot; ΣV is the sum of the averages of the pots of each test lot; $\bar{V} = \Sigma V/n$ is the average of the averages (V) of the pots of each test lot; ΣV^2 is the sum of the squares of the averages of each pot of a test lot; and $\bar{V}\Sigma V$ is the product of $\bar{V}$ and ΣV. The value of t was calculated according to the equation

$$t = \frac{\bar{V}_1 - \bar{V}_2}{SE}.$$

The values of SE and t were determined for various pairs of test lots from the data of the next to the last reading of experiments 6 and 7. Detailed data are given in table 3 and figures 2 and 3 for experiment 7 only; in this experiment the differences noted in bud averages per test lot were great enough to be statistically significant.

Counts were made only of the grossly detectable buds. If they had been made on sectioned material with a microscope, the values for buds present would have been higher and the intervals after treatment until buds were first recorded would have been shorter.

Samples of hypocotyls were collected, fixed, imbedded, sectioned, and stained according to the methods reported in our earlier paper (6). The sections were studied to determine the effects of the application of lanolin and of indoleacetic acid in lanolin on the behavior of epidermal cells of the upper half of the hypocotyl with reference to the cell divisions which initiate bud primordium formation, and upon the development of primordia present in the lower half of the hypocotyl at the time of decapitation and treatment.

Observations and findings

In the decapitated hypocotyl of flax the initial gross injury (the cut) is succeeded by the following train of injury effects: the decapitated member generally guttates for a period whose length depends upon the physiologic state of the hypocotyl prior to decapitation and upon external conditions following it; it shrinks, evident in decrease of its diameter and, at times, even of its length; it becomes less green; the cut surface begins to dry out and becomes necrotic, the depth to which the underlying tissues are involved depending upon the physiologic state of the hypocotyl prior to the primary injury and upon external conditions following it. In vigorous hypocotyls recovery from the injury and injury effects attendant to decapitation is marked by prompt reattainment of the green color and of the diameter of the predecapitation hypocotyl; by early cessation of desiccation and necrosis of the cut and of the tissues below it; by prompt cicatrization of the wound; and by slight tumor formation immediately below the healing surface through development of an inner wound tissue (interior callus). After this the hypocotyl experiences a limited general diameter increase, and, in time, buds become grossly evident on most hypocotyls (fig. 1*D*).

If a hypocotyl succeeds in healing the wound promptly and develops a shoot, this shoot generally attains only one-half

the length of the main axis of a nondecapitated plant, but its blooming lags only a few days behind that of the nondecapitated plant. Since such a plant does not develop the cotyledonary axes of the normal plant, even in recovery it still is abnormal and pathic in that its production of the fiber-bearing members and its production of flowers and fruit are far behind that of the normal plant. The primary injury and its effects leave a lasting, irreversible effect even under the most favorable circumstances.

If the hypocotyls are less vigorous when decapitated and external conditions are not favorable, the decapitation injury and its effects may terminate fatally in many hypocotyls. The older the hypocotyl at time of decapitation, the greater is the chance of death of the pathic hypocotyl. Hypocotyls of plants grown in soil are more pathic and die more readily than those grown in sand and supplied with mineral nutrients. The pathic state of a decapitated hypocotyl may be brief, ending promptly in death, or prolonged, terminating in death only after some weeks. Development of hypocotyledonary buds and shoots does not necessarily insure survival of the injured hypocotyl.

These tests, therefore (as all so-called "physiological" tests with plants which experimentally are injured physically or chemically as well as all so-called "pathological" tests with plants which experimentally are injured infectiously), are made with plants experiencing, and reacting to, an injury—hence, with plants in a pathic state. In the decapitated, untreated hypocotyl this state is not complicated by addition of lanolin or by indoleacetic acid in lanolin. Hence decapitated but untreated hypocotyls were run as controls for the decapitated lanolin controls. The pathology of the decapitated untreated and treated hypocotyl in recovery, and in failure of recovery, will be described in detail in a later paper.

Table 1 reports some of the results of experiments 1–5, which were conducted at various times of the year with decapitated controls, lanolin-treated decapitated controls, and with decapitated hypocotyls treated with various concentrations of indoleacetic acid. The bud counts of these experiments are not given here because of their large number. Table 3 for experiment 7 shows the great variability of such data. Table 1 also reports the number of surviving plants of experiments 1 and 2.

Part of experiment 1 was planned to determine whether lanolin alone and indoleacetic acid in lanolin affect hypocotyledonary bud formation and development in decapitated hypocotyls of flax and to give a clue as to the maximum concentration of indoleacetic acid usable. It was found that one treatment with (*a*) 3% or 1% indoleacetic acid completely inhibited bud formation for the period of bud readings from April 27 to May 16; (*b*) 0.3% or 1:1 (0.116%) indoleacetic acid produced temporary inhibition, the former concentration producing the longer inhibition; (*c*) 1:2 (0.058%) indoleacetic acid produced no inhibition at all. In addition to their effects upon bud formation, concentrations 3%, 1%, and 0.3% produced pathic changes such as temporary blanching of the hypocotyl and excessive swelling directly below the site of application (fig. 1*B*). Furthermore, these pathic effects terminated fatally. Thus in experiments 1 and 2, one month after the last bud count, 99%, 100%, and 70%, respectively, of the plants treated with 3%, 1%, and 0.3% indoleacetic acid had died. These lethal effects are similar to the herbicidal effects

TABLE 1

EFFECTS OF LANOLIN AND INDOLEACETIC ACID UPON BUD FORMATION BY DECAPITATED HYPOCOTYLS OF FLAX AND UPON THEIR SURVIVAL

Experiment no.	History of plants and of treatment	Kind of treatment	Response of hypocotyl	
1.....	Planted 4/15	Decapitated	Buds on 6th day	100% alive
	Up 4/19	" , lanolin	"	100% "
	Treated 4/21	" , 3% indoleacetic acid	No buds on 19th day	99% dead
	Read 4/27, 4/29	" , 1% "	"	100% "
	5/2, 5/4, 5/9, 5/16	" , 0.3% "	No buds on 14th day; buds on 19th day	70% "
		" , 1:1 (0.116%) "	No buds on 6th day; buds on 8th day	100% alive
		" , 1:2 "	Buds on 6th day	100% "
2.....	Planted 4/26	Decapitated	Buds on 6th day	100% alive
	Up 4/29	" , lanolin	"	100% "
	Treated 5/6	" , 1% indoleacetic acid	No buds on 19th day	100% dead
	Read 5/12, 5/14	" , 0.3% "	"	100% "
	5/16, 5/18, 5/20	" , 1:1 "	No buds on 10th day; buds on 12th day	100% alive
	5/23, 7/11	" , 1:2 "	Buds on 6th day	100% "
3.....	Planted 5/20	Decapitated	Buds on 5th day	
	Up 5/24	" , lanolin	"	
	Treated 6/3	" 1:1 indoleacetic acid	No buds on 14th day; buds on 21st day	
	Thereafter daily	" , 1:2 "	"	"
	applications of	" , 1:4 "	"	"
	lanolin and indole-	" , 1:8 "	No buds on 12th day; buds on 14th day	
	acetic acid	" , 1:16 "	No buds on 7th day; buds on 10th day	
	Read 6/8, 6/10,	" , 1:32 "	No buds on 5th day; buds on 7th day	
	6/13, 6/15, 6/24,	" , 1:64 "	Buds on 5th day	
	7/5	" , 1:128 "	No buds on 5th day; buds on 7th day	
4.....	Planted 9/19	Decapitated	Buds on 7th day	
	Up 9/25	" , lanolin	Buds on 7th day	
	Treated 10/4	" , 1:1 indoleacetic acid	No buds on 20th day	
	Thereafter daily	" , 1:2 "	"	
	applications of	" , 1:4 "	"	
	lanolin and of	" , 1:8 "	"	
	indoleacetic acid	" , 1:16 "	"	
	Read 10/11, 10/13,	" , 1:32 "	Buds on 7th day	
	10/15, 10/17,	" , 1:64 "	"	
	10/19, 10/24	" , 1:128 to 1:524,288 inclusive	"	
5.....	Planted 9/29	Decapitated	Buds on 17th day	
	Up 10/4	" , lanolin once daily	"	
	Treated 10/20	" , 1:1 indoleacetic acid once daily	"	
	Thereafter one-half of each lot daily applications of lanolin and of indoleacetic acid	" , 1:4 " once daily	No buds on 26th day	
		" , 1:16 " once daily	"	
		" , 1:64 to 1:262,144, inclusive once daily	"	
	Read 10/27, 10/29, 10/31, 11/2, 11/5		Buds on 17th day	
			No buds on 26th day	
			Buds on 17th day	
			"	

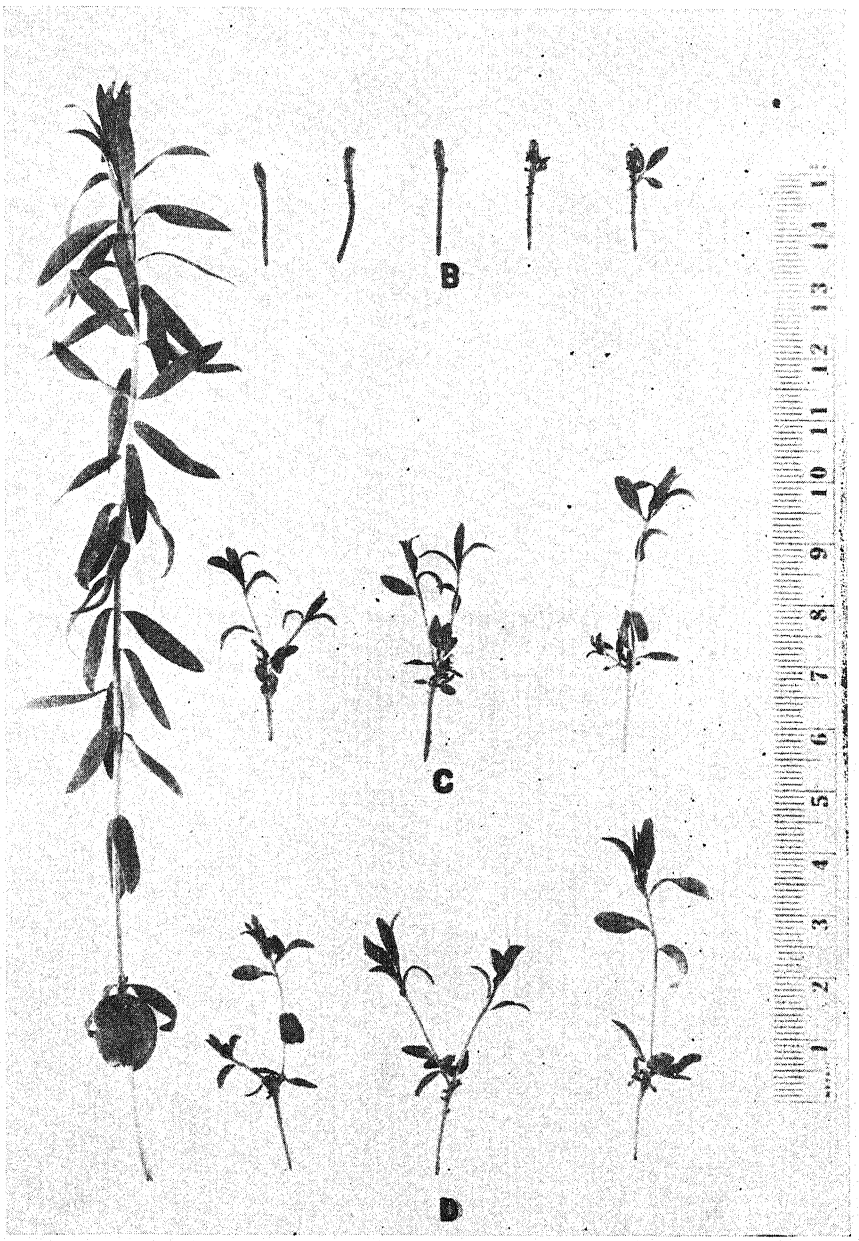


FIG. 1.—All plants, of same age from seed planted in quartz sand April 15, had experienced same cultural conditions when severed on May 10, at ground line, for photographing. *A*, *left*, representative control plant showing hypocotyl, cotyledons, short first internode, longer higher internodes; cotyledonary buds beginning to develop into axes. All axes began to flower June 7; buds in leaf axils also had then become grossly detectable. Note that hypocotyl in *A* is longer than those of *B*, *C*, and *D*. Sets *B*, *C*, and *D* are hypocotyls from plants decapitated directly below cotyledonary node on April 21 and recut on April 25, the day of treatment (experiment 1, table 2). *B*, treated with 0.3% indoleacetic acid in lanolin; *C*, treated with 1:1 (0.116%)

(Legend continued on following page)

noted for bean and tomato by LINK, WILCOX, and LINK (4). All untreated controls and all lanolin-treated decapitated plants alive at the time of treatment had survived.

Another part of experiment 1 was designed to determine the effect of a delay of 2 and 4 days in treatment of the decapitated hypocotyls with lanolin and with high concentrations of indoleacetic acid. The plants were decapitated on April 21. One half were not cut again but were treated on the 2d and 4th days after decapitation. The other half were divided into three lots. The first of these was treated immediately after decapitation; the second, 2 days, and the third, 4 days, after decapitation (fig. 1). Presence or absence of buds is shown in table 2 for the days on which readings were made.

The results indicate that concentrations of 3%, 1%, 0.3%, and 0.116% indoleacetic acid were more effective in inhibiting or retarding bud formation if applied on the day of decapitation than on later days. The acid seemed to be equally effective whether applied to a new cut or to the old cut surface.

Concentrations of 3% and 1% indoleacetic acid applied on the day of decapitation completely inhibited bud formation until the plants died. A concentration of 0.3% applied on that day delayed it for 18 days, but the plants died. Applied 2 and 4 days after decapitation, 3% and 1% indoleacetic acid delayed bud formation for 13 days. Because of the findings of this test, treatment in all subsequent experiments was applied on the day of decapitation.

Experiment 2 was a repetition of part of experiment 1 with elimination of the treatment with 3% indoleacetic acid. The results were essentially those obtained in experiment 1 as to effects on budding and on death of the hypocotyl.

In experiment 3 the hypocotyls were treated daily with lanolin and with indoleacetic acid, and higher dilutions of indoleacetic acid were used—1:1 (0.116%) to 1:128 (0.0009%). The plants were grown during the period of maximum sunshine (June 3–July 5). In addition to verifying the results of experiments 1 and 2, the data of this experiment indicate that repeated applications of indoleacetic acid in the higher dilutions were more effective in producing inhibition of bud formation than a single application of the same concentration. Thus concentration 1:32 (0.0036%) indoleacetic acid applied daily produced a 10-day retardation of grossly detectable bud formation, whereas a single application of concentration 1:2 (0.058%) in experiments 1 and 2 did not produce any retardation or inhibition.

Experiment 4 was a duplication of ex-

(Continued from preceding page)

indoleacetic acid in lanolin; *D*, treated with lanolin paste and used as controls. Note substitute axes in *D;* when more than one developed, one became dominant. It flowered a few days later than axes of uncut plant but was shorter and more slender. Also note additional buds in center plant. Plants treated with lanolin immediately and 2 days after decapitation resembled these plants. In other experiments, concentrations of 0.3% and 1:1 indoleacetic acid, when applied immediately after decapitation, completely and permanently inhibited initiation and development of bud primordia and bud and shoot development in upper and lower halves of hypocotyl. Concentration 1:128 (0.0009%) was highest dilution which significantly retarded bud initiation and development (experiment 1, table 3 and figs. 2, 3). Shoots of set *C* are shorter than those of set *D*. Note club-shaped tumefaction of hypototyl in set *B;* complete absence of buds in specimen to left and increasing numbers of buds scattered along entire length of hypocotyl, as well as small shoots in other specimens. These buds did not appear until 14 days after treatment, a delay of 12 days, and then appeared only in 30% of plants. These hypocotyls died prematurely, as did those treated with 1:1 indoleacetic acid. Note in table 2 that 0.3% indoleacetic acid produced complete and permanent inhibition in hypocotyls treated on date of decapitation.

periment 3 and, in addition, tested dilutions of indoleacetic acid up to and including 1:524,288 (0.0000012%). The light conditions were not so favorable as in experiment 3, but the results were essentially the same.

In experiment 5 the effects of one and of daily treatments were tested concurrently. Light conditions were not so favorable as in experiment 4, but the findings corroborate those of experiments 3 and 4. In the higher concentrations—1:1 (0.116%) and 1:4 (0.029%) indoleacetic acid—the single treatment and the daily treatment produced the same effects. Concentration 1:16 (0.00725%) indoleacetic acid showed a differential effect, the daily treatments delaying bud formation longer than the single treatment.

Perusal of the results of experiments 1–5 indicates that the effectiveness of a particular concentration within the maximum range of effectiveness varied slightly in the different experiments. The time of bud appearance in the controls and lanolin-treated plants ranged from 5 (3d experiment [May 20–July 5]) to 17 days (5th experiment [September 29–November 5]). Plants treated with 1:1 (0.116%) indoleacetic acid did not produce buds until the 8th day in experiment 1 (April 27–May 16) and not until the 12th day in experiment 2 (May 12–July 11). Buds appeared by the 21st day in experiment 3 (June 8–July 5) in plants treated daily with concentration 1:4 (0.029%), whereas in experiment 5 the same treatment held buds back beyond the 26th day. In this test, concentration 1:16 (0.00725%) held back buds past the 26th day, whereas in experiment 3 this

TABLE 2

PRESENCE OR ABSENCE OF BUDS FOLLOWING DECAPITATION AND DEFERRED TREATMENT AND FOLLOWING DECAPITATION WITH REPEATED CUTTING AND IMMEDIATE TREATMENT

Date of treatment	Date of reading	Kind of treatment											
		Decapitation 4/21; new cut on day of treatment						Decapitation cut of 4/21					
		Control	Lanolin	Indoleacetic acid				Control	Lanolin	Indoleacetic acid			
				3%	1%	0.3%	1:1			3%	1%	0.3%	1:1
4/21	4/27	+	+	o	o	o	o	.....	.....	.....	.....	.....	.....
	4/29	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/2	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/4	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/9	+	+	o	o	+	+	.....	.....	.....	.....	.....	.....
4/23	4/27	+	+	o	o	o	o	+	+	o	o	o	o
	4/29	+	+	o	o	o	+	+	+	o	o	o	+
	5/2	+	+	o	o	+	+	+	+	o	o	o	+
	5/4	+	+	o	o	+	+	+	+	o	o	+	+
	5/9	+	+	+	+	+	+	+	+	+	+	+	+
4/25	4/27	+	+	o	o	o	+	+	+	o	o	o	+
	4/29	+	+	o	o	o	+	+	+	o	o	o	+
	5/2	+	+	o	o	+	+	+	+	o	o	+	+
	5/4	+	+	o	o	+	+	+	+	o	o	+	+
	5/9	+	+	+	+	+	+	+	+	+	+	+	+

concentration retarded them only 7–9 days. These results probably indicate that the metabolic status of the plant is a factor in determining the effectiveness of various concentrations of indoleacetic acid (2, 3, 6) in affecting bud formation and development.

Some data of experiments 1–5 and of other experiments indicated at times that lanolin possibly affects the number of buds formed. They also indicated that treatments immediately after decapitation and after a lapse of 1–6 hours, respectively, produced differences in the number of buds formed and also that a single treatment either immediately after decapitation or after some hours on the same day produced different results than did a series of treatments on successive days.

The data of experiments 1–5 also had indicated the possibility that concentrations of indoleacetic acid ranging from 1:256 (0.00045%) to 1:524,288 (0.0000012%) at times increased the number of buds formed and developed by the treated hypocotyl. To determine whether the differences noted in the average of buds formed were statistically significant, experiment 6 was set up. It included the following eleven test lots, each comprising five pots with twenty plants each: (1) cut, untreated control; (2) lanolin, one treatment applied immediately after decapitation; (3) lanolin, one treatment applied 6 hours after decapitation; (4) lanolin, first treatment applied immediately after decapitation, thereafter daily; (5) lanolin, first treatment applied 6 hours after decapitation, thereafter daily; (6) indoleacetic acid 1:4096 (0.000028%), one application immediately after decapitation; (7) indoleacetic acid 1:4096, one application 6 hours after decapitation; (8) indoleacetic acid 1:4096, first application immediately after decapitation, thereafter daily; (9) indoleacetic acid 1:4096, first application 6 hours after decapitation, thereafter daily; (10) indoleacetic acid 1:8192 (0.000014%), one application immediately after decapitation; and (11) indoleacetic acid 1:16,384 (0.000007%), one application immediately after decapitation. This experiment was conducted from March 7 to April 10. Readings were made on the 3d, 5th, 9th, and 15th days after buds first had begun to appear. Readings of the number of buds counted on the 9th day were treated statistically. No statistical significance was found for the differences noted between test lots 1 and 2–11, inclusive; 2 and 3; 4 and 5; 6 and 7; 8 and 9; 6 and 8; and 7 and 9. This indicates that the differences noted were due to individual variation in the flax plants tested and that the flax plant and the methods used are not suitable to determine whether indoleacetic acid in certain concentrations stimulates bud formation and development in the decapitated hypocotyl.

The preliminary experiments and experiments 1–5 also had indicated that certain higher concentrations of indoleacetic acid—1:1 (0.116%) to 1:256 (0.00045%)—which are not high enough completely to inhibit bud formation and development, are adequate to reduce the number of buds formed per plant. The threshold seems to vary with the metabolic state of the plant.

Experiment 7 was set up so that statistically usable data might be obtained to determine the validity of these indications. The experiment included the five following test lots, each comprising six pots of twenty plants each: (1) cut, untreated control; (2) lanolin, one treatment; (3) indoleacetic acid 1:1 (0.116%), one treatment; (4) indoleacetic acid 1:128 (0.0009%), one treatment; and

(5) indoleacetic acid 1:4096 (0.000028%), one treatment. Lanolin and indoleacetic acid were applied immediately after decapitation. Readings were begun 7 days after treatment and were made again on the 9th, 11th, 15th, and 21st days. The data obtained on the 15th day were treated statistically. Table 3 records the data of all readings in terms of percentage of plants budded and of average number of buds per plant. Figure 2 shows the percentage of plants budded and figure 3 the average number of buds per plant for all readings.

Calculation of SE and t showed that the differences between the cut, untreated control (1) and the lanolin-treated decapitated hypocotyl (2) are not statisti-

TABLE 3

VARIABILITY IN RATE OF BUD FORMATION BY HYPOCOTYLS OF FLAX DECAPITATED AND TREATED ON FEBRUARY 22

Date of reading	Pot no.	Decapitated		Lanolin		Indoleacetic acid 1:4096		Indoleacetic acid 1:128		Indoleacetic acid 1:1	
		Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant
3/1.....	1	23.5	0.41	8.6	0.21	16.6	0.25	0	0	0	0
	2	18.1	0.27	26.3	0.36	33.3	0.38	0	0	0	0
	3	15.7	0.21	31.2	0.62	4.76	0.09	0	0	0	0
	4	5.5	0.05	4.34	0.04	11.1	0.11	0	0	0	0
	5	20.0	0.20	4.76	0.14	16.6	0.16	0	0	0	0
	6	21.7	0.26	10.00	0.10	13.6	0.13	0	0	0	0
	1–6	17.6	0.23	13.1	0.22	16.1	0.19	0	0	0	0
3/3.....	1	47.0	1.41	73.9	2.21	66.6	1.79	31.5	0.42	0	0
	2	72.7	1.95	89.4	3.21	66.6	2.28	86.1	0.86	0	0
	3	36.8	1.05	81.2	2.37	47.6	1.14	5.0	0.05	0	0
	4	44.4	1.00	82.6	2.65	66.6	2.16	0	0	0	0
	5	45.0	0.95	52.3	1.04	61.1	2.05	0	0	0	0
	6	47.8	1.00	65.0	1.44	59.0	1.77	0	0	0	0
	1–6	49.5	1.23	73.7	2.13	61.2	1.85	6.6	0.08	0	0
3/5.....	1	94.1	3.41	91.3	3.91	81.5	3.91	68.4	2.78	0	0
	2	100.0	3.77	94.7	5.00	100.0	3.57	100.0	3.34	0	0
	3	84.2	2.84	93.7	5.62	95.2	2.90	55.0	1.20	0	0
	4	83.3	2.33	91.3	4.56	100.0	3.22	79.1	2.00	0	0
	5	89.4	3.47	90.4	2.66	88.8	3.83	52.1	1.30	0	0
	6	91.3	3.04	90.0	3.35	86.3	3.86	40.7	1.22	0	0
	1–6	91.5	3.16	91.7	4.12	92.7	3.56	65.4	1.94	0	0
3/9.....	1	100.0	9.23	100.0	7.81	95.8	7.08	89.4	3.52	0	0
	2	100.0	9.40	100.0	8.36	100.0	7.47	100.0	5.60	0	0
	3	100.0	9.26	100.0	8.62	100.0	4.76	95.0	2.45	0	0
	4	100.0	8.88	100.0	9.47	94.7	6.05	95.8	3.54	0	0
	5	100.0	9.00	100.0	6.19	100.0	6.27	86.3	2.72	0	0
	6	100.0	7.73	100.0	6.95	95.4	7.45	85.1	3.14	0	0
	1–6	100.0	8.88	100.0	7.90	97.6	6.55	91.8	3.59	0	0
3/15.....	1	100.0	10.47	100.0	8.13	100.0	8.25	89.4	3.88	0	0
	2	100.0	10.09	100.0	9.00	100.0	8.70	100.0	6.08	0	0
	3	100.0	10.15	100.0	9.31	100.0	6.29	100.0	3.10	0	0
	4	100.0	10.11	100.0	10.26	100.0	7.50	95.8	4.58	0	0
	5	100.0	9.80	100.0	7.04	100.0	7.77	86.3	2.95	0	0
	6	100.0	9.50	100.0	7.60	100.0	9.18	92.5	3.70	0	0
	1–6	100.0	10.00	100.0	8.55	100.0	7.97	94.47	4.08	0	0

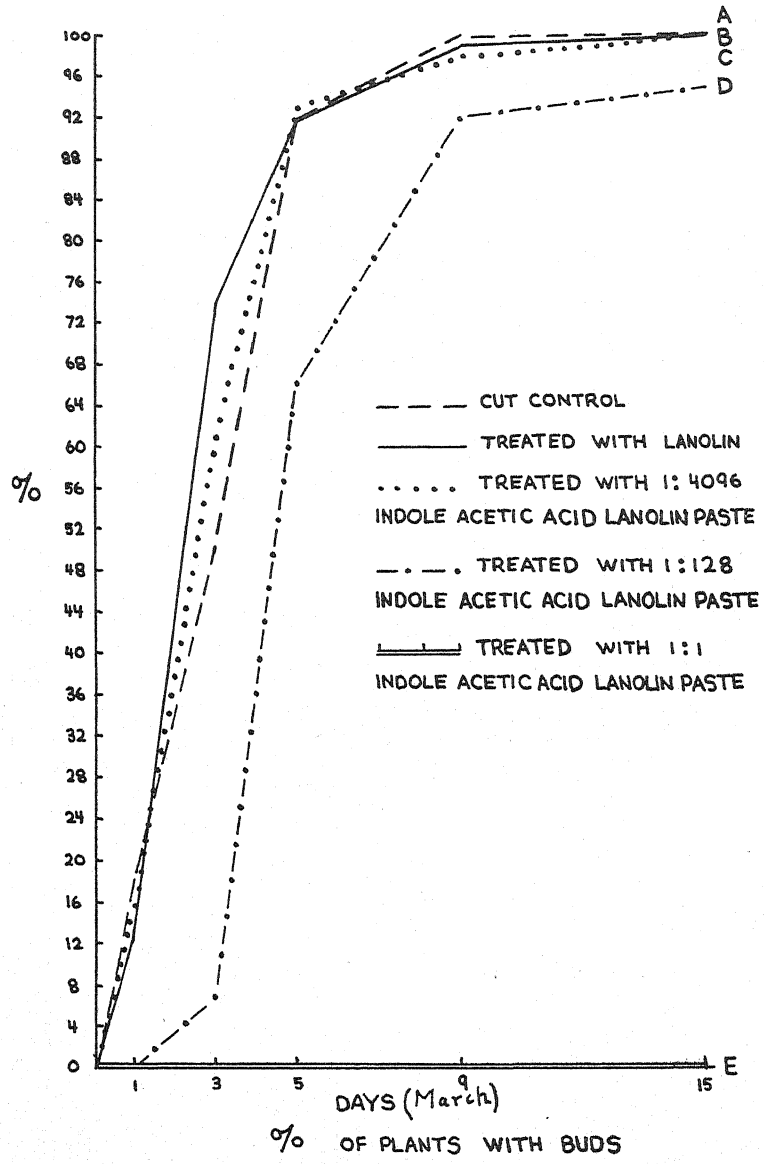


FIG. 2.—Percentage of plants with buds based on counts made March 1, 3, 5, 9, and 15, and tabulated in table 3. *A*, cut, untreated control plants; *B*, decapitated lanolin-treated control plant; *C*, decapitated plants, treated with 1:4096 (0.000028%) indoleacetic acid–lanolin; *D*, decapitated plants, treated with 1:128 (0.0009%) indoleacetic acid–lanolin; *E*, decapitated plants, treated with 1:1 (0.116%) indoleacetic acid–lanolin.

cally significant; that the differences between the lanolin-treated hypocotyls (2) and those treated with indoleacetic acid 1:128 (0.0009%) (4) are highly significant.

The findings of this experiment corroborate those of experiment 6 to the effect that lanolin treatment produces no significant difference in the rate and amount of budding in the treated plant as compared with the cut, untreated plant; that indoleacetic acid in greater dilution than 1:128 (0.0009%) produces no significant changes in the number of buds formed; and that indoleacetic acid 1:1 (0.116%) may produce complete inhibition of bud formation.

When hypocotyls treated with various

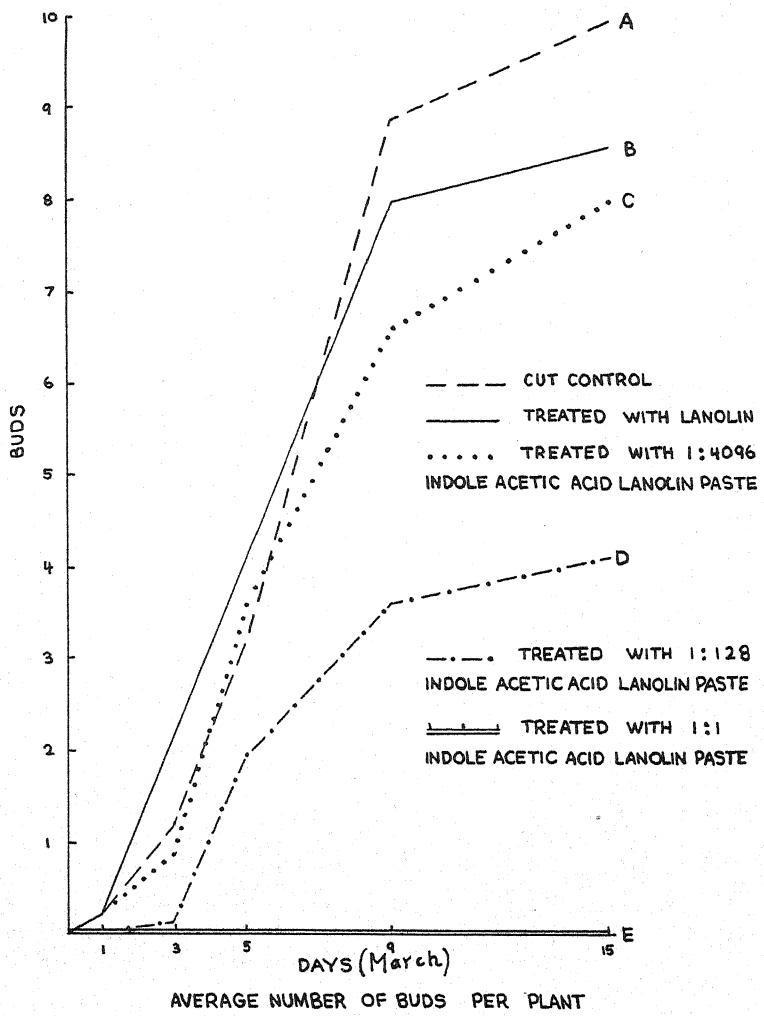


FIG. 3.—Average number of grossly detectable buds per plant, based on counts made March 1, 3, 5, 9, and 15 and tabulated in table 3. *A*, *B*, *C*, *D*, and *E* represent same plants as in fig. 2. Differences between *A* and *B*, *A* and *C*, and *B* and *C* on March 9 are not statistically significant; differences between *A* and *D*, *A* and *E*, *B* and *D*, and *B* and *E* are.

concentrations of indoleacetic acid were examined histologically, it was found that, in those instances in which indoleacetic acid produces complete inhibition of grossly visible buds, the initiation of bud primordia in the upper half of the hypocotyl is completely inhibited and the development of the primordia present in its lower half also is completely inhibited. In those instances in which formation and development of buds are temporarily inhibited and retarded by indoleacetic acid, these effects are due to temporary inhibition in the initiation of bud primordia in the upper half of the treated hypocotyl and to temporary inhibition of development of the primordia present in its lower half.

Discussion

It is evident from the results of the experiments that indoleacetic acid could serve in the intact flax plant as an agent in the inhibition of (*a*) bud primordium initiation; (*b*) development of the primordium into a bud; and (*c*) development of the buds into shoots. Tests to determine whether auxins are present in the flax plant and whether they are so distributed as to serve in inhibition of hypocotyledonary bud primordia in the intact plant are the subject of researches to be reported in other papers.

VAN OVERBEEK (7) reported that 0.1% indoleacetic acid in lanolin applied to the decapitated pea plant completely inhibited development of the lateral buds. In some of our experiments 0.116% indoleacetic acid completely inhibited formation of hypocotyledonary bud primordia and of their development into buds and, of these, into shoots. Concentrations higher than this (i.e., 0.3%–3%) also inhibited these events, but they also led to premature death of the treated organ after stimulating it to excessive tumor formation beneath the treated wound.

Our failure to establish whether any of the concentrations of indoleacetic acid tested incites or hastens bud primordia formation and development of these into buds may indicate that, in addition to auxins, factors other than indoleacetic acid—and not controlled in our tests—also play a role in bud primordium initiation and in bud development.

The results of our experiments show that indoleacetic acid and presumably the auxins of the flax plant so affect the epidermal cells of the hypocotyl that they do not experience the transverse cell divisions characteristic of bud primordium initiation or the subsequent divisions and other cell processes characteristic of development of the primordium into a bud and of development of the bud into a shoot. They do not, however, indicate how these effects are brought about. Presumably they could be affected directly or indirectly.

It is evident that the effects are quite definite or specific so far as cell division is concerned; while cell divisions characteristic of bud primordium initiation are inhibited, cell divisions involved in formation or development of wound tissue are not inhibited.

The fact that repeated applications of a given concentration of indoleacetic acid are more effective than one application in inhibiting bud formation and development indicates that attainment and maintenance of a certain threshold value is essential for continued inhibition of bud initiation and development and that the indoleacetic acid applied is used up, or inactivated. The fact that a certain concentration, unless renewed, inhibits bud formation for only a limited time points to the same conclusion.

Delay for more than a day in treatment with a concentration which is inhibitory if applied immediately retards but does not completely inhibit bud formation. This suggests that this given concentration of indoleacetic acid more readily inhibits the epidermal cell division involved in bud primordium initiation than it does the processes involved in development of the primordium into a bud and of the bud into a shoot.

There are many studies dealing with the inhibitory effects of auxins upon the development of lateral buds, and several theories about the action of auxin in producing this effect, which have been critically evaluated by VAN OVERBEEK (7). We are aware, however, of only one report on the effects of auxins upon initiation of buds which are not mediated by callus tissue. BEAL (1) reported that *Lilium harrisii*, when decapitated and treated on the cut surface with 3% indoleacetic acid in lanolin, initiated buds in the axils of leaves which normally do not bear buds. He described the course of events as follows:

> In *L. harrisii* the region of visible response to the treatment is limited largely to the cells of the epidermis and outer cortex in the immediate vicinity of the leaf axil. The epidermal cells of the stem immediately above the axil elongate radially, while the outer cortical cells centripetal to, as well as slightly below, them enlarge and in the course of about 5 days begin dividing. This marks the initiation of the region at which buds will later develop.

At the time we undertook the experiments reported here, we entertained the hypothesis that a range of concentration might be found which would stimulate the epidermis of the decapitated flax hypocotyl to initiate buds. For a time, as pointed out above (5), it seemed as though we had found, in certain experiments, concentrations of indoleacetic acid which did produce this effect. Had this finding been substantiated, we should be dealing with a situation in which an auxin (indoleacetic acid) induces cell division without first inducing cell enlargement. Possibly no concentration of indoleacetic acid plays a role in inciting this train of events. Unfortunately, either the great variation in capacity of individual hypocotyls to initiate buds, or the essentially uncontrolled metabolic status of our plants, or both, made it impossible to obtain statistically significant data bearing upon the question of whether indoleacetic acid stimulates epidermal cells to divide transversely and thus to begin the initiation of bud primordia. The findings show clearly, however, that a range of concentration of indoleacetic acid exists which inhibits and retards the cell divisions which initiate buds in the decapitated hypocotyl of flax.

We are not concerned here with the interesting questions as to whether this effect of auxin is brought about directly or indirectly and what the mechanism is whereby it is brought about. These questions will be discussed in later papers.

The findings indicate that an auxinic mechanism may be a method whereby initiation and subsequent development of hypocotyledonary bud primordia are controlled in the intact flax plant. Studies of the auxinic situation in the intact flax plant, and in isolated members of it, in its possible relation to bud inhibition will be reported on in later papers.

The finding that for a given concentration of indoleacetic acid, in any particular experiment, repeated applications are more effective than a single application may indicate that the auxin applied is used up or bound in some way and that

repeated applications are essential for maintenance of the threshold concentrations necessary to inhibit bud initiation and development. These thresholds seem to vary with the metabolic or nutritional status of the plant prior to and following decapitation and treatment.

Summary

1. The decapitated hypocotyl of flax, with its initial injury and the resultant injury effects, is in a pathic state during the course of the experiments reported here. The metabolic status of the hypocotyl at the moment of infliction of the injury, and the external conditions to which it is exposed after its mutilation, determine to what extent the injury effects terminate in recovery and shoot regeneration, or in death.

2. Single or repeated applications of lanolin alone to the wound of the decapitated flax hypocotyl produced no detectable effects upon survival of the hypocotyl or upon its initiation and development of hypocotyledonary buds.

3. Applications of 3% and of 1% indoleacetic acid in lanolin to the decapitation wound completely and permanently inhibited bud primordium development in the lower half, and bud primordium initiation in the upper half, of the hypocotyl. The treated plants died earlier than the untreated cut controls, as reported earlier for bean and tomato.

4. Application of 0.3% indoleacetic acid in lanolin retarded these bud events for a long time. It also led to the premature death of most of the treated hypocotyls. Application of 1:1 (0.116%) indoleacetic acid in lanolin led to slight retardation or to complete inhibition of these bud events, depending upon the number of treatments and upon the metabolic status of the tested plants.

5. The lowest concentration of indoleacetic acid noted to retard bud primordium initiation and development was 1:128 (0.0009%). One treatment with this concentration in February was more effective than repeated treatments in May.

6. While one treatment with 1:16 (0.007%) concentration of indoleacetic acid retarded grossly detectable bud appearance for 17 days, repeated treatment suppressed it completely for 26 days.

7. In the higher concentrations, 1:4 (0.029%) and above, no difference was noted in the effect of single as compared with repeated treatments.

8. Under the conditions of the experiment no concentration was found which stimulated bud primordium initiation and development.

9. The findings show that indoleacetic acid in lanolin may retard or completely inhibit the transverse divisions of epidermal cells of the hypocotyl which are the first detectable beginning of hypocotyledonary bud primordium initiation in flax.

10. The greater efficiency of repeated applications than of a single application of the same concentration of indoleacetic acid in inhibiting or retarding these events may indicate that the former treatment maintains the threshold necessary for inhibition by replacing auxin used up or bound in the hypocotyl.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
AND
MOUNT SINAI HOSPITAL
CHICAGO, ILLINOIS

LITERATURE CITED

1. BEAL, JOHN. Histological responses of three species of *Lilium* to indoleacetic acid. BOT. GAZ. **99**: 881–911. 1938.
2. BURNS, G. P., and HEDDEN, M. E. Conditions influencing regeneration of the hypocotyl. Bot. Centbl. Beihefte I **19**:383–392. 1906.
3. EGGERS, VIRGINIA. Influence of carbohydrates and nitrate-nitrogen nutrition on development of hypocotyledonary buds in flax. BOT. GAZ. **107**: 385–390. 1946.
4. LINK, GEORGE K. K.; WILCOX, HAZEL; and LINK, ADELINE DE SALE. Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extract, β-indoleacetic acid and wounding. BOT. GAZ. **98**:816–867. 1937.
5. LINK, GEORGE K. K., and EGGERS, VIRGINIA. Inhibition of adventitious bud initiation in hypocotyls of flax by indoleacetic acid and flax extract. Nature **142**:398. 1938.
6. ———, and ———. Mode, site, and time of initiation of hypocotyledonary bud primordia in *Linum usitatissimum* L. BOT. GAZ. **107**:441–454. 1946.
7. VAN OVERBEEK, J. Auxin distribution in seedlings and its bearing on the problem of bud inhibition. BOT. GAZ. **100**:133–167. 1938.

MEGASPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF ALLIUM CERNUUM

JOHN B. MURPHY

Introduction

During recent years much previous work on the Liliaceae has been questioned. Reinvestigation has, in some cases, effected grave changes in the pattern of the life-story of members of the family. The *Lilium* type of embryo sac, established by TREUB and MELLINK (36) as a result of their study of *Lilium bulbiferum*, has lately been eliminated because of the subsequent reinvestigation by BAMBACIONI (1, 2, 3). Investigators such as GUIGNARD (11), SARGANT (31), COULTER (7), and MOTTIER (25) had observed pecularities in the development of the *Lilium* megagametophyte, but considered these to be abnormalities and failed to investigate them any further. BAMBACIONI established that the mature embryo sac of *L. bulbiferum* followed the pattern of the *Fritillaria* type as first described in her report on *F. persica* and *L. candidum*. COOPER (5, 6) suggested that the *Fritillaria* type is probably characteristic of the whole genus *Lilium*.

The present investigation was undertaken to make a comparative study of megasporogenesis and embryo-sac development in a genus related to *Lilium* with special attention to the type of embryo sac and its possible variation from the traditional type. An uninvestigated species, *Allium cernuum* Roth, was considered a favorable species for such study.

Material and methods

Ovaries were collected at intervals over a period extending from June through August in the years 1943 and 1944. They were prepared for sectioning by the usual paraffin technique. Some were stained with safranin and fast green; others were stained with safranin, gentian violet, and orange G in clove oil. Sections cut at 8 or 10 μ were found most suitable for study.

Observations

DEVELOPMENT OF THE MEGASPOROPHYLLS

Three carpel primordia appear as crescent-shaped mounds in the flattened area of the flower summit inside the inner whorl of stamens and alternate with

them. The bases of the primordia grow more rapidly than the apices, thus causing the megasporophylls to be leaflike in appearance, with a broad base and a bluntly pointed apex. The subsequent growth of the carpels results in syncarpy by an inward arching of the three segments until eventually the inturned margins come in direct contact with each other; at the point of contact the carpels fuse, forming the style primordium. The primordium of the compound pistil grows upward, resulting in formation of a simple, persistent, threadlike style arising from the tip of the ovary.

The basal portions of the carpels constitute the ovary. At about the time that the style initial begins to elongate, the ovule primordia differentiate from the inner ridges of the carpellary margins in the ovarian portion, thus showing axillary placentation.

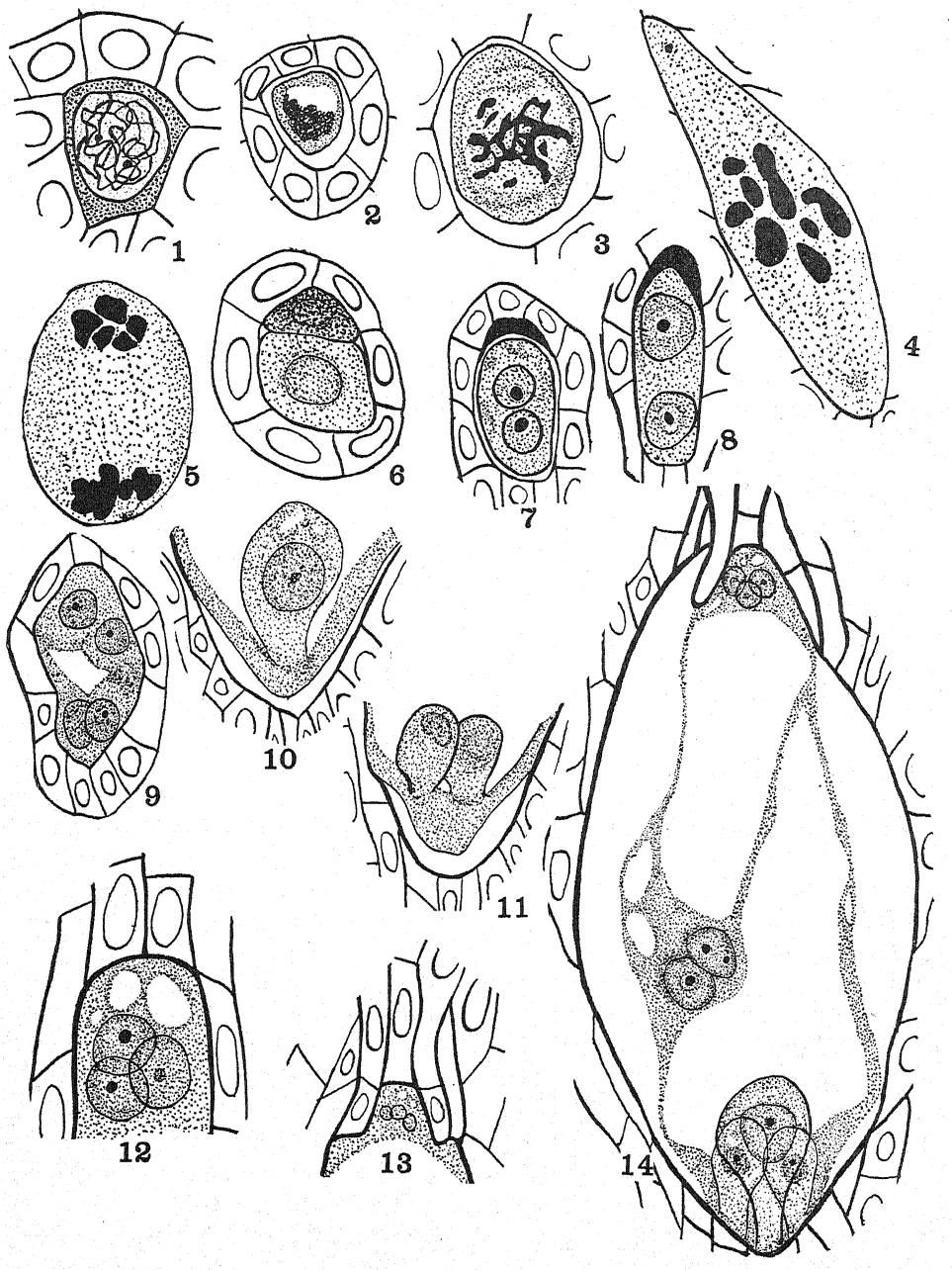
Development of the megasporangium

The ovule initial appears as a dome-shaped mass of cells which arises from the placenta at the base of the carpel. Periclinal divisions of cells of the hypodermal layer of the sporophyll result in the formation of a mass of nucellar tissue. The overlying epidermal cells divide only anticlinally, thus compensating for the enlargement of the nucellus and, at the same time, forming a layer of epidermal tissue. The nucellus develops rapidly, and, before the style is very far developed, a single hypodermal archesporial cell is differentiated. Shortly after the archesporium is formed, the inner integument arises by periclinal cell division as an annular fold of the epidermis. The inner integument develops rapidly, and, by the time the archesporium is in synapsis, the outer integument has also appeared and is considerably elongated. The integuments grow with greater rapidity than the nucellar tissue and completely envelop the ovule before meiosis is completed. The inner integument varies from three to five rows of cells in thickness at maturity and is slightly shorter than the outer integument, which is seven to nine cell rows thick. Both integuments are tightly pressed together, but at the time of pollination the cells of the integuments contract, resulting in a micropylar opening. After the entrance of the pollen tube the integuments again become tightly pressed together.

During early stages of the megagametophyte the ovule undergoes a complete inversion, resulting in its anatropous orientation. In the process of anthesis there is a rapid growth of the chalazal tissue, and the funiculus becomes sharply curved just below the chalaza; as a result, the integuments are inverted and lie alongside the stalk of the funiculus, with the micropyle directed toward the placenta. The integuments reach maturity shortly before anthesis is completed.

Development of the megagametophyte

The archesporium differentiates about 3 or 4 days before the flowers emerge from the bud, which is about the time that the floral envelope starts turning to its white or rose color. The archesporial cell (fig. 1) forms before the initials of the inner integument appear and functions directly as the megaspore mother cell. The megaspore mother cell undergoes the first reduction division, resulting in two homomorphic dyads (fig. 6). Phases of this division are seen in figures 1–6. Judging by the few sections found in the dyad stage, it may be concluded that this stage is very brief. After the first meiotic division the micropylar dyad begins to



FIGS. 1–14.—Camera lucida drawings. In figures 1–9 the micropylar end of megagametophyte is toward top of page. Fig. 1, cross-section of megaspore mother cell, early prophase (950×). Fig. 2, megaspore mother cell, synizesis (440×). Figs. 3–5, late prophase, metaphase, and anaphase of heterotypic division (950×). Fig. 6, late stage of dyads. Micropylar dyad beginning to disintegrate (950×). Fig. 7, two-nucleate megagametophyte. Micropylar dyad nearly disintegrated (440×). Fig. 8, older two-nucleate stage, nuclei migrating to opposite ends of sac (440×). Fig. 9, reconstruction from two sections, four-nucleate stage of megagametophyte. Micropylar megaspore completely disintegrated (440×). Fig. 10, transverse section of egg cell (950×). Fig. 11, orientation of two synergid cells (950×). Fig. 12, cross-section through antipodal cells and heavily stained nucellar cell walls (950×). Fig. 13, disintegrating antipodal cells and heavily stained chalazal nucellar cell walls (950×). Fig. 14, reconstruction from three sections, mature eight-nucleate megagametophyte (950×).

disorganize, apparently without any further nuclear division (figs. 6, 7, 8). The chalazal dyad enlarges, forms a new achromatic figure, and undergoes a second reduction division, resulting in two homomorphic megaspore nuclei which migrate to the opposite ends of the cell (figs. 7, 8). The cell does not form a cross-wall. The binucleate megaspore stage lasts relatively long. Both of these megaspores take part in the formation of the embryo sac, thus placing it in the bisporic "*Allium* type." The disintegrating dyad is still visible at this stage (fig. 8), although the residue is rapidly being digested and assimilated by the surrounding cells.

After the second meiotic division small vacuoles begin to appear, and the megagametophyte enlarges to almost twice its original size (fig. 8). Both megaspore nuclei now divide again, giving rise to the four-nucleate stage (fig. 9), two nuclei being oriented at each end of the cell. Following this division, the megagametophyte becomes increasingly vacuolated, almost doubling the dimensions of the two-nucleate stage (fig. 9). The central portion of the sac is practically one large vacuole. The nuclei at each end of the sac are side by side in the same plane though not touching each other. The micropylar nuclei, however, are not in the same plane as the chalazal nuclei. Figure 9 is a composite of the two planes. The disintegrating dyad has been completely absorbed by the time this stage is reached. The four-nucleate stage is apparently of short duration Each of these four nuclei undergoes a second mitosis, resulting in the formation of an eight-nucleate megagametophyte. For a short while it continues to increase in size, primarily as the result of vacuolization (fig. 14).

One nucleus migrates from each end of the sac toward the central peripheral region, where they approximate each other (fig. 14). These polar nuclei are about equal in size and chromatic density. The three nuclei remaining in the micropylar end organize into a group of three cells (fig. 14). The egg cell is much larger than the synergids and is pear shaped. The synergid cells (figs. 11, 14) are about equal in size and density and lie behind the egg. One of the synergids seems to disintegrate shortly before fertilization.

The antipodals organize into cells and form a rather loose mass in the chalazal region (fig. 14). In some cases they are about equal in size and density and may persist throughout the maturation of the megagametophyte (fig. 12), but more frequently they are very small (fig. 13) or may even be absent.

After the orientation of the nuclei the megagametophyte grows rapidly to its mature size (fig. 14). Cytoplasmic vacuolation causes most of the cytoplasm to be massed at the micropylar end around the egg and synergids. A smaller amount of dense cytoplasm is gathered around the polar nuclei, and a still smaller amount lies at the chalazal end surrounding the antipodals (fig. 14). Small vacuoles are often found between the antipodals and the end of the sac (figs. 12, 13).

The mature megagametophytes examined were about 198 μ in length and 98 μ in width, being narrow and more pointed at the chalazal end of the sac. The antipodal region of the protoplasm is wedged as a small pocket into the surrounding tissue (fig. 13). The megagametophyte is mature about 6–12 hours after the opening of the flower, and fertilization occurs within 3 days after the flower bud opens.

CHROMOSOMES.—The number of chromosomes in the megagametophyte was determined as eight (fig. 4).

NUCELLUS

The nucellar cells adjacent to the megagametophyte are somewhat different from the others. As the archesporium differentiates, the inner nucellar cells divide periclinally, forming a distinct layer, the walls of which thicken as the megagametophyte develops. As the megagametophyte reaches maturity and its nuclei become oriented, the walls of the surrounding cells attain their greatest thickness at the chalazal and micropylar ends. The cells immediately surrounding the chalazal end of the megagametophyte are distinctly larger, thicker walled, and differently shaped than the other nucellar cells. They are long, blunt at one end, and taper toward the megagametophyte, which seems to be wedged into these cells (figs. 12, 13, 14). The antipodals are generally oriented in such a way as to be harbored under the pointed ends of these projecting cells (figs. 13, 14). The inner walls of the nucellar cells surrounding the micropylar end of the megagametophyte are also unusually thick and of characteristic shape (figs. 10, 14). The walls of the nucellar cells along the rest of the megagametophyte are also slightly thicker than usual (fig. 14). The wall thickening of the nucellar cells surrounding the megamametophyte is noticeably diminished after fertilization.

Discussion

Although a number of variations have been reported in the development of the megagametophyte of several species of *Allium*, this investigation shows that *A. cernuum* follows the characteristic bisporic *Allium* type (formerly called the "Scilla type") as first described by STRASGURGER (34) for *A. fistulosum*. This investigation shows, moreover, that the chalazal dyad undergoes three successive divisions to form the eight-nucleate megagametophyte, whereas the micropylar dyad disintegrates without further division. SCHURHOFF (33), MODILEWSKI (23, 24), and JONES and EMSWELLER (16) made similar observations in other species of *Allium*. WEBER (37) noted that the megagametophyte frequently originated from the micropylar dyad in many of the species that she investigated. MESSERI (22) observed that a large number of the megagametophytes belonged to the monomegasporal type. PORTER (28), in a recent description of *A. mutabile* Michx., stated that the chalazal dyad undergoes another division on forming two megaspores of which the chalazal one functions. This would place the megagametophyte of *A. mutabile* in the normal monosporic type. In the present investigation particular attention was focused on *A. cernuum* for evidence of a similar occurence, but no such observation was made. The evidence of PORTER and of MESSERI seems incomplete and demands a thorough reinvestigation.

Many megagametophytes included under the *Allium* type are reported to have less than the usual eight nuclei. MESSERI considered it sufficiently common in the several species she studied to establish a "seven-nucleate" *Allium* type. The limitation occurs in the chalazal end of the megagametophyte after the four-nucleate stage. No such observation was made in this investigation, and in every case the megagametophyte contained the typical eight nuclei. The "egglike" antipodal cells observed by MESSERI in *A. subhirsutum* and *A.*

schoenoprasum, and by MODILEWSKI in *A. nigrum*, were not found in *A. cernuum*. On the contrary, this investigation confirms the report of ELMORE (10) that the mature gametophyte shows a more or less marked reduction of the antipodal apparatus and, in many cases, even a complete loss of antipodals.

There are two synergids in *A. cernuum*. They are about equal in size and density in contrast with the one large and one small synergid observed by JONES and EMSWELLER in *A. cepa*, or with only the one synergid observed by PORTER in *A. mutabile* Michx. A "filamentous apparatus" in the synergids, as reported by MESSERI, was not observed in *A. cernuum*, nor was one of the synergids hypertrophied, as found in *A. cepa* (16). One of the synergids, however, showed rapid disintegration just prior to fertilization. The statement of PORTER that the egg and synergid are sister cells and that the polar nuclei are also sister cells finds no confirmation in this study. However, the thick-walled cells of the inner nucellus, as reported in *A. mutabile* Michx. (28), are characteristic of *A. cernuum*.

The author was interested in the theory that a species of *Allium* might possibly belong to the *Fritillaria* type as described by BAMBACIONI and by COOPER for many species of *Lilium*. *Allium cernuum* does not, however, deviate from the usual *Allium* type.

A comparative study of megagametophyte development in other genera of the *Allium* type offers an interesting variation. HEATLY (13), in *Trillium cernuum*, MCALLISTER (20), in *Streptopus roseus*, and MCKENNEY (21), in *Scilla hyacinthoides* var. *caerules* and *S. campanulata* found that the embryo sac develops from the micropylar dyad. SVENSSON (35) observed that the micropylar dyad starts to divide in *Lycopsis arvensis* but degenerates before the division is completed. In *S. nonscripta*, as described by HOARE (14), the micropylar dyad functions and the chalazal dyad disintegrates, although it first undergoes a division forming an "antigone" consisting of four nuclei. The Alismaceae, as reported by JOHRI (15) and by DAHLGREN (8, 9), show a reduction in the chalazal part of the embryo sac, which is commonly six-nucleate. The nuclei divide normally up to the four-nucleate stage, after which the chalazal pair remain undivided and the micropylar pair divide once again.

Instead of the three divisions usually found in the *Allium* type, MAGNUS (17) observed only two divisions from the chalazal megaspore of *Podostemon subulatus*. Subsequent studies by HAMMOND (12), WENT (38, 39, 40), and MAHESHWARI (19) question the existence of a *Podostemon* type. SCHNARF (32) likewise questions the megagametophyte described by MAGNUS in *Dicraea elongata*, in which he reported one division of the micropylar dyad, forming the egg and one synergid, and one anticlinal division of the chalazal dyad, forming two antipodal cells. MAGNUS stated that no polar nucleus exists and that consequently double fertilization is impossible.

PACE (26), describing *Cypripedium spectabile*, *C. parviflorum*, *C. pubescens*, and *C. candidum*, found that the megagametophyte was derived from the chalazal dyad. She further observed a four-nucleate megagametophyte resulting from two divisions with a pair of nuclei at each end of the embryo sac. The micropylar nuclei form the two synergids and the two chalazal nuclei form the egg and a single polar nucleus. At the time of fertilization one of the synergid nuclei takes part in triple fusion. PALM (27) established the "*Cypripedium* type" on the

basis of PACE's investigation. RUTGERS (30), in a criticism based primarily upon certain figures in PACE's work, pointed out several weaknesses of the *Cypripedium* type. Recent publications by PROSINA (29), investigating *C. guttatum*, and CARLSON (4), in her study of *C. parviflorum*, show that the development of the megagametophyte is bisporic and of the usual *Allium* type, with imperfect development at the chalazal end so that there are usually only five or six nuclei in the embryo sac. MAHESHWARI (18), in a review of the types of embryo sacs in angiosperms, included a complete list of the investigations on many other genera of the *Allium* type.

Summary

1. The megasporangium of *Allium cernuum* is essentially like that of *Lilium*.

2. The archesporium arises directly from a hypodermal cell about 3 or 4 days before the flowers emerge from the bud.

3. The megagametophyte is formed from the chalazal dyad. The micropylar dyad disintegrates without further division.

4. The chalazal dyad undergoes three divisions, resulting in an eight-nucleate megagametophyte.

5. The mature megagametophyte consists of a large vacuolated egg, two smaller synergids—one of which usually disintegrates before fertilization—two polar nuclei of equal size, and three antipodal cells which may or may not persist.

6. The haploid number of chromosomes in *A. cernuum* is eight.

DE PAUL UNIVERSITY
CHICAGO, ILLINOIS

LITERATURE CITED

1. BAMBACIONI, V. Ricerche sulla ecologia e sulla embriologia di *Fritillaria persica* L. Ann. Bot., Roma, 18:7–37. 1928.
2. ———. Contributo alla embriologia di *Lilium candidum* L. R. C. Accad. Lincei. 8:612–618. 1928.
3. ———. Nuove ricerche sull'embriologia delle Gigliaceae. Ann. Bot., Roma, 19:365–382. 1931.
4. CARLSON, M. Megasporogenesis and development of the embryo sac of *Cypripedium parviflorum*. BOT. GAZ. 107:107–114. 1945.
5. COOPER, D. C. Development of the embryo sac of *Lilium Henyri*. Proc. Nat. Acad. Sci. 20: 163–166. 1934.
6. ———. Macrosporogenesis and development of the embryo sac of *Lilium Henyri*. BOT. GAZ. 97: 346–355. 1935.
7. COULTER, J. M. Contribution to the life history of *Lilium Philadelphicum* L. I. The embryo sac and associated structures. BOT. GAZ. 23:413–422. 1897.
8. DAHLGREN, K. V. Die Embryologie einiger Alismataceae. Svensk bot. Tidskr. 22:1–17. 1928.
9. ———. Die Embryosackentwicklung von *Echinodorus macrophyllus* und *Sagitarria sagittifolia*. Planta 21:602–612. 1934.
10. ELMORE, C. J. Some results from the study of *Allium*. BOT. GAZ. 26:277–278. 1898.
11. GUIGNARD, L. Nouvelles études sur la fécondation. Ann. Sci. nat. Bot., Ser. 7, 14:163–296. 1891.
12. HAMMOND, S. L. Development of *Podostemon ceratophyllum*. Bull. Torrey Bot. Club. 64:17–36. 1937.
13. HEATLY, M. A study of the life history of *Trillium cernuum* L. BOT. GAZ. 61:425–429. 1916.
14. HOARE, G. Gametogenesis and fertilization in *Scilla nonscripta*. La Cellule 42:269–292. 1933.
15. JOHRI, B. M. Studies in the family Alismaceae IV. *Alisma plantago* L.; *Alisma plantago aquatica* L.; *Sagittaria graminea* Mich. Proc. Ind. Acad. Sci. B, 4:128–138. 1936.
16. JONES, H. A., and EMSWELLER, S. L. Development of the flower and macrogametophyte of *Allium cepa*. Hilgardia 10:415–428. 1936.
17. MAGNUS, W. Die atypische Embryonalentwicklung der Podostemaceen. Flora, Jena, 105:275–336. 1913.
18. MAHESHWARI, P. A critical review of the types of embryo-sacs in angiosperms. New Phytol. 36:359–417. 1937.
19. ———. Recent work on the types of embryo-sacs in angiosperms—a critical review. Jour. Indian Bot. Soc. 20:229–261. 1941.
20. MCALLISTER, F. The development of the embryo sac in the Convallariaceae. BOT. GAZ. 58:137–153. 1914.

21. McKENNEY, R. E. Observations on the development of some embryo sacs. Contrib. Bot. Lab. Univ. Pennsylvania 2:80–86. 1898.
22. MESSERI, A. Ricerche embriologiche e cariologiche sopra i generi *Allium* e *Nothoscordon*. Nuovo Giorn. Bot. ital., n.s., 38:409–441. 1931.
23. MODILEWSKI, J. Zur Kenntnis der Polyembryonie von *Allium odorum* L. Bull. Jard. bot. Kieff 7–8:65–70. 1925.
24. ———. Die Embryobildung bei *Allium odorum* L. Bull. Jard. bot. Kieff 12–13:27–48. 1931.
25. MOTTIER, D. M. Über das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung. Jahrb. wiss. Bot. 31:125–158. 1898.
26. PACE, L. Fertilization in *Cypripedium*. BOT. GAZ. 44:353–374. 1907.
27. PALM, B. Studien über Konstrucktionstypen und Entwicklungswege des Embryosackes der Angiospermen. Diss. Stockholm. 1915.
28. PORTER, T. R. Development of the megagametophyte and embryo of *Allium mutabile*. BOT. GAZ. 98:317–327. 1936.
29. PROSINA, M. N. Über die von Cypripedium—Typus abweichende Embryosackentwicklung von *Cypripedium guttatum*. Sw. Planta 12:532–544. 1931.
30. RUTGERS, F. L. Reliquiae Treubianae. III. Embryo sac and embryo of *Moringa oleifera*. The female gametophyte of angiosperms. Ann. Jard. bot. Buitenz. 33:1–66. 1923.
31. SARGANT, E. Direct nuclear division in the embryo sac of *Lilium martagon*. Ann. Bot., Lond., 10:107–109. 1896.
32. SCHNARF, K. Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. 2:565–585. 1936.
33. SCHÜRHOFF, P. N. Zur Polyembryonie von *Allium odorum*. Ber. deut. bot. Ges. 40:374–381. 1922.
34. STRASBURGER, E. Die Angiospermen und die Gymnospermen. Gustav Fischer, Jena. 1879.
35. SVENSSON, H. G. Zur Embryologie der Hydrophyllaceen, Borraginaceen, und Heliotropiaceen. Diss. Upsala. 1925.
36. TREUB, M., and MELLINK, J. Notice sur le développement du sac embryonnaire dans quelques Angiospermes. Arch. néerl. Sci. 5: 452–457. 1880.
37. WEBER, E. Entwicklungsgeschichtliche Untersuchungen über die Gattung *Allium*. Bot. Arch. 25:1–44. 1929.
38. WENT, F. Untersuchungen über *Podostomaceen*. I. Verh. Akad. Wet., Amst., Sec. II. 16:(1). 1910.
39. ———. Untersuchungen über *Podostomaceen*. II. Verh. Akad. Wet., Amst., Sec. II. 17:(2). 1912.
40. ———. Untersuchungen über *Podostomaceen*. II. Verh. Akad. Wet., Amst., Sec. II. 25:(1). 1926.

FLOWER-BUD FORMATION AND DEVELOPMENT IN MANGIFERA INDICA[1]

MARGARET J. MUSTARD AND S. J. LYNCH

Introduction

Most of the better varieties of mangoes (*Mangifera indica* L.) fail to produce good crops every year. In attempts to overcome this problem, various cultural treatments aimed at inducing flower formation have been employed (1, 3, 8). SEN and MALLIK (6) recently determined the time of flower-bud differentiation for one variety of Indian-grown mango, the Langra, and emphasized the importance of this knowledge in connection with the problem of irregular bearing. SEN (7) has utilized this information in preparing a "tentative cultural schedule" as an aid to the more regular production of these fruits.

The present investigation was undertaken to determine the time of flower-bud differentiation and to study the subsequent floral development in some varieties of mangoes grown in southern Florida.

Material and methods

Collections of material were made for two consecutive years from trees of Haden, Brooks, and Cambodiana varie-

[1] This investigation was conducted by the authors while both were employed by the University of Florida at the Sub-Tropical Experiment Station at Homestead, Florida.

ties and a turpentine seedling mango. These four trees are located on soil of the Rockdale series in southern Dade County. Although different parts of the same tree were not always at the same stage of development, an effort was made to select buds which seemed to be representative of the average stage on each date. Collections were started the first year, on September 10, 1943, and were continued at 2-week intervals thereafter until each tree came into bloom. The following year the first collections were made on September 23 and were continued over a similar period.

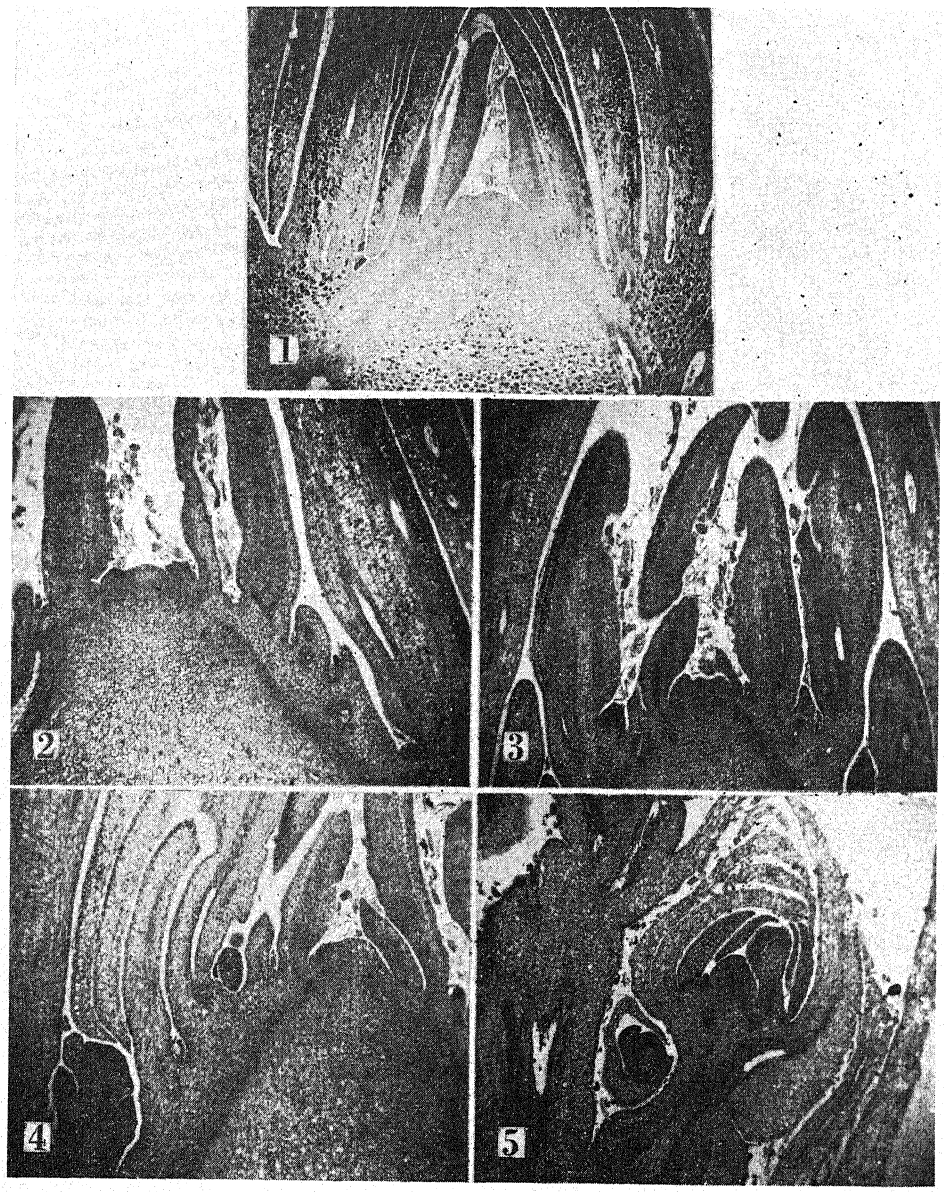
The buds were fixed in F.A.A., dehydrated in tertiary butyl alcohol, and imbedded in paraffin. Before sectioning, the imbedded material was softened for 1–2 weeks in an alcohol-glycerin mixture. Sections were made at 10 μ. Safranin and fast green were used in staining the mounted serial sections.

Observations

A mature mango inflorescence consists of a main axis and a number of profusely branched secondary axes (fig. 6). Each secondary axis, the ramifications of such axes, and the pedicels are subtended by bracts. An individual inflorescence may bear as many as four thousand flowers (3), of which the ratio of bisexual to staminate varies with the variety, the bisexual flowers usually being in a decided minority.

Prior to floral differentiation a mango bud consists of a cone of meristematic tissue surrounded by the outer bud scales (fig. 1). The inner bud scales project as finger-like protuberances from the surface of this cone of tissue. The earliest indication of floral development (6) is the appearance of mounds of meristematic tissue in the axils of these inner bud scales or bracts (fig. 2). Meristematic areas in the axils of the more proximal bud scales are usually the first to begin floral differentiation. As the season progresses and such growing points enlarge, it becomes obvious that each is the primordium of a secondary axis (fig. 3). Soon these secondary axes give rise to outgrowths of tissue (fig. 4) which prove to be bracts, in the axils of which other small mounds of meristematic tissue develop. These and subsequently differentiated growing-points may follow either of two courses of development: (1) they may differentiate into additional axes, thus continuing the ramification of the inflorescence; or (2), they may differentiate into floral primordia (fig. 5). If such a mound of tissue differentiates into another axis, the process is repeated until a growing-point is formed which differentiates into a flower primordium. It appears that the position of such growing-points on the inflorescence governs to some extent their future course of development, since the greatest degree of ramification occurs in the proximal portions of the lower secondary axes.

In order to record these progressive stages of floral development as they were observed in the sectioned material, a method was employed similar to that described by SELL and his co-workers (5). The numerical ratings of 1 through 5 were assigned to each of the progressive stages of development shown in figures 1–5. Using these figures as a basis, it was possible to determine and record numerically the approximate stage of development of an individual bud. Figures for the average stage of development presented in table 1 were obtained by multiplying the number of buds found to be at each of these stages by the assigned numerical rating, adding the products, and dividing this figure by the total num-



FIGS. 1–5.—Longitudinal sections of mango buds. Fig. 1, prior to differentiation. Fig. 2, very early stage of floral differentiation, small protrusion in axil of bud scale appearing at lower right. Fig. 3, slightly more advanced stage, enlarged axillary protrusions or primordia of secondary axes. Fig. 4, early differentiation of bracts on a secondary axis. Fig. 5, arrangement of individual flower parts of hermaphroditic flower.

ber of buds collected on that date. Thus, for example, of the five Haden buds collected on 9/10/43, one was found to be at stage 1 (fig. 1) and four at stage 2 (fig. 2); therefore, the average stage of floral development on that date was 1.8.

It is evident from table 1 that floral differentiation began slightly earlier in the Haden, Cambodiana, and Brooks in 1943 than in 1944; whereas in the turpentine seedling it seems to have occurred at approximately the same time both years. Such variation is to be expected, however, since the time of initiation of floral differentiation is dependent upon a combination of environmental factors and tree condition. These results indicate that by the end of October of both years the majority of the terminal buds studied had shown signs of floral differentiation. Between the time of flower-bud differentiation and that of inflorescence expansion, development seems to have progressed without a period of dormancy such as is common among the deciduous fruit trees of the North Temperate Zone.

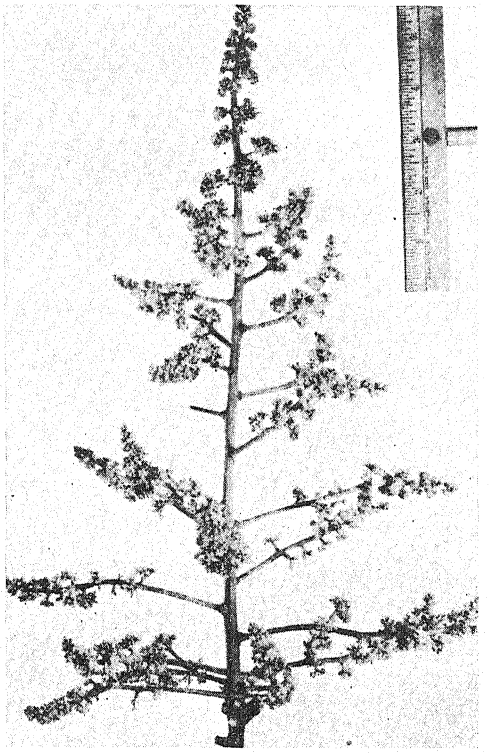


FIG. 6.—Mature inflorescence

TABLE 1

AVERAGE STAGE OF FLORAL DEVELOPMENT OF TERMINAL MANGO BUDS. INDIVIDUAL BUDS NUMERICALLY RATED BY COMPARISON WITH FIGURES 1–5

Variety	Average stage of development								
	9/10/43	9/25/43	10/9/43	10/23/43	11/6/43	11/18/43	12/4/43	12/18/43	12/31/43
Haden	1.8	1.8	1.3	1.0	2.0	1.0	2.0	2.0	2.0
Cambodiana	2.0	2.0	2.0	1.5	2.5	2.7	.....	.....	.....
Brooks	1.3	2.0	2.0	2.0	2.3	2.2	3.5	5.0	.....
Turpentine	1.5	2.0	1.5	1.7	3.5	3.0	.....	.....	.....
		9/23/44	10/7/44	10/20/44	11/4/44	11/17/44	12/1/44	12/13/44	12/30/44
Haden	.....	1.0	1.0	1.0	1.3	2.0	2.0	2.7	4.0
Cambodiana	.....	1.0	1.7	1.7	1.5	2.0	1.0	3.3	.....
Brooks	.....	1.0	1.0	2.0	1.7	2.0	2.0	.....	.....
Turpentine	.....	1.5	1.5	2.0	2.7	3.0	4.0	2.0	.....

These observations on the time of floral differentiation agree closely with those made by SEN and MALLIK. These authors found that it occurs shortly after the end of September in the Langra mango grown in India. STURROCK (9) previously estimated the time of floral differentiation as occurring in October or early November in some varieties of Florida-grown mangoes. His collections were too late in the season to determine the early stages of differentiation. REECE (4) observed a similar lack of dormancy between the time of floral differentiation and maturation of flowers in Florida-grown avocados.

Summary

1. Floral differentiation occurred shortly before the end of October in the four varieties of mangoes studied.

2. No period of dormancy occurred between the time of floral differentiation and that of inflorescence expansion.

CHEMISTRY DEPARTMENT
UNIVERSITY OF MIAMI
CORAL GABLES, FLORIDA
AND
FLORIDA LIME AND AVOCADO GROWERS
PRINCETON, FLORIDA

LITERATURE CITED

1. ALCALA, PROCESO E., and SAN PEDRO, A. Bud differentiation in smudged mango trees. Philippine Agr. 24:27–48. 1935–36.
2. JULIANO, JOSÉ B., and CUEVAS, NUMERIANO. Floral morphology of the mango (*Mangifera indica* Linn.) with special reference to the Pico variety from the Philippines. Philippine Agr. 21: 449–473. 1932.
3. POPENOE, WILSON. Manual of tropical and subtropical fruits, pp. 79–146. Macmillan Co., New York. 1920.
4. REECE, P. C. Differentiation of avocado blossom buds in Florida. BOT. GAZ. 104:323–328. 1942.
5. SELL, H. M., TAYLOR, H. A., and POTTER, G. F. Effect of chemical treatments in prolonging dormancy of tung buds. II. BOT. GAZ. 106:215–223. 1944.
6. SEN, P. K., and MALLIK, P. C. The differentiation of the flower-bud of the mango. Ind. Jour. Agr. Sci. 11 (Part I):74–79. 1941.
7. ———. Irregular bearing of mango. Indian Farming 5:408–411. 1944.
8. STURROCK, DAVID. Notes on the mango, pp. 40–53. Stuart Daily News. 1944.
9. STURROCK, T. T. Flower-bud formation in the mango. University of Florida. August, 1943. (Unpublished.)

CURRENT LITERATURE

Pure Cultures of Algae: Their Preparation and Maintenance. By E. G. PRINGSHEIM. Cambridge: At the University Press; New York: Macmillan Co., 1946. Pp. xii+119. $1.75.

For more than a quarter of a century PRINGSHEIM has been the leader in study of algae by pure culture methods. The extensive series of algae which he has isolated include many differing markedly in their growth requirements when grown *in vitro*. In his *Pure Cultures of Algae* he passes along the benefit of his long experience in this field of botanical investigation. His book is not a laboratory manual of the "cookbook" type but is a connected and integrated running account of how to obtain cultures of algae. The reviewer recommends that the person wishing to learn about methods of cultivating algae should begin by reading this small book from cover to cover. By doing this, one gets the concentrated essence of PRINGSHEIM's long experience and thus avoids many pitfalls that would otherwise be encountered the hard way.

The author points out the great advantage of preparatory cultures in which one attempts to gain an insight into the conditions suitable for the existence and multiplication of particular algae. Enrichment (selective) cultures are especially valuable for this purpose, since, by varying the composition of the medium, pH, temperature, and other environmental factors, growth of certain algae in the inoculum is favored, while growth of other algae is checked. Following the chapter on preparatory cultures there are chapters on media, on methods of plating, on isolation by means of micropipettes, and on the maintenance of cultures. The concluding chapter deals with the general conditions essential for growth of members of each of the larger taxonomic groups of algae.

There are some books in which one copy in the departmental library is sufficient. Here is one in which this is not the case; every man who has use for it will want a copy in his personal library.—GILBERT M. SMITH.

Chromosome Atlas of Cultivated Plants. By C. D. DARLINGTON and E. K. JANAKI AMMAL. London: Allen & Unwin, Ltd.; New York: Macmillan Co., 1945. Pp. 397. $2.75.

The chromosome numbers of some ten thousand species of the most useful plants and of their wild relatives and ancestors have been assembled in this world list.

In an introduction, consisting of three parts, Part I deals with the origin of cultivated plants as to (*a*) the foundations as determined especially by VON HUMBOLDT, DE CANDOLLE, DARWIN, and MENDEL; (*b*) the centers of origin according to VAVILOV; (*c*) evolution by cultivation; and (*d*) the invention of plant breeding. Part II introduces the chromosomes, and Part III covers the use and meaning of chromosome numbers.

Next is given an explanation of the catalogue. The families of angiosperms follow the order and bear the numbers of HUTCHINSON's *Families of Flowering Plants*, with 233 of the 332 families represented. Two exceptions to this order are noted. Because of their chromosome affinities, the tribes Allieae and Agapantheae have been retained in the heterogeneous Liliaceae and the Nolanaceae have been kept separate from the Convolvulaceae. The families of gymnosperms, taken from ENGLER and GILG's *Syllabus der Pflanzenfamilien*, follow the author's own order, while the tribes of Pinaceae follow HUTCHINSON's unpublished classification. Five columns are employed in the catalogue. The first column is used for the generic and specific names of some two thousand genera with more than eleven thousand species listed. The second column gives the popular names, the third the chromosome numbers, the fourth the author from whose paper the numbers are reported, and the fifth column the uses to which the plants are put. The catalogue, covering about 309 pages, comes next, followed by a bibliography and by an index to the families and genera.

No attempt is made to cite all the literature dealing with chromosome numbers. Where two accounts differ in number for the same species, both are quoted, apart from certain ancient errors; but, where accounts agree, the last important reference only is quoted, since from this the earlier counts can usually be obtained.

The book is an important contribution and will be highly valuable for teachers and investigators in all phases of economic plant production.—J. M. BEAL.

VARIATION IN ECOTYPES OF POA PRATENSIS[1]

D. C. SMITH,[2] E. L. NIELSEN,[3] AND H. L. AHLGREN[4]

Introduction

The existence of apomictic seed development appears to be a fact well established for *Poa pratensis* L. Though apomixis is the usual form of reproduction, many plants may produce seed frequently following amphimixis. The species is highly polymorphic, and extensive investigations have been made to determine the extent and nature of the variability. The possibilities of selection of superior plants and of their subsequent propagation as improved strains have been recognized. Commercial seed of Kentucky bluegrass is heterogeneous, and progenies from it may give a wide complex of plant types. The method of harvesting commercial seed and the possible selection for seed production characters rather than for vegetative vigor may be accompanied by a diminished pasture value. In most areas of the Northeastern and North Central states, however, the species is now sufficiently prevalent to be considered as naturalized, and it is seldom seeded for pasture purposes.

Breeding investigations with Kentucky bluegrass were begun at the Wisconsin Experiment Station in 1936. The principal objectives have been (*a*) the isolation and testing of superior biotypes for pasture and turf purposes, (*b*) the testing of the breeding behavior of such biotypes, and (*c*) the determination of the nature of variation in the species.

The present paper presents the results of studies of variation in several characters. A large number of seeded panicles for use in progeny studies was collected in Wisconsin and other states where the grass is relatively common.

Review of literature

BRITTINGHAM (4) has comprehensively reviewed and discussed the earlier literature. Several workers have studied apomixis, but not all have presented results of progeny tests. ÅKERBERG (1) found that various selected families segregated from 5.9 to 13.4% sexual (aberrant) plants. Thirty-seven of forty-four native collections from Sweden, Germany, and Norway were "practically" constant morphologically, the remaining seven each showing great variation. ÅKERBERG (2) also studied families from collections made in Sweden, Norway, Finland, Denmark, Germany, and England. Five to ten plants of each family were grown. Seventy-nine families averaged 3.9% aberrant. Within each of nine families no two plants were alike, while each of five families was essentially homogeneous.

[1] Contribution no. 204 from the Department of Agronomy, University of Wisconsin, Madison, Wis., in co-operation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Department of Agriculture. Published by permission of the Director of the Wisconsin Agricultural Experiment Station.

[2, 4] Professor of Agronomy, Wisconsin Agricultural Experiment Station; [3] Associate Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Department of Agriculture. The writers are indebted to the Statistical Laboratory of the Wisconsin Experiment Station for aid in the statistical calculations and to R. J. MUCKENHIRN for counsel regarding the soil classifications utilized.

TINNEY and AAMODT (14) investigated 102 progenies numbering 21 to 243 plants each—a total of 11,696 plants. The strains were of domestic and foreign origin as follows:

Wisconsin, 31	Sweden, 6
Virginia, 1	Denmark, 1
Missouri, 2	England, 1
Ottawa, Canada, 34	India, 1
Guelph, Canada, 21	Russia, 2
Norway, 2	

The average percentage of aberrants for all progenies was 1.6, with values ranging from 0 to 21.9%. Forty-eight progenies appeared to be uniform. The thirty-one Wisconsin collections averaged 1.65% aberrant, while the fourteen other nonuniform progenies averaged 3.65% aberrant.

BROWN (6) found that progenies of six lots of selected plants produced 0.09–18.18% aberrant types. Five others were apparently uniform. Fifty-five to 133 plants of each lot were grown.

BRITTINGHAM (4) observed an average of 14.8% aberrants in 115 progenies—approximately 10,000 individuals—resulting from open pollination. Four of the 115 progenies showed complete uniformity; one had the maximum of 90.5% aberrants. This material originated from permanent pastures, commercial seed lots, numbered experiment-station strains, and foreign-plant introductions.

EDGECOMBE (8) studied twenty-nine progenies of *P. pratensis;* all but one were considered to be uniform or nearly so. MUSGRAVE (11) concluded that about 90% of all types of *P. pratensis* observed reproduced seed apomictically.

BROWN (5) gave a brief summary of the taxonomic status of *P. pratensis*, indicating that, since 1811, eleven classifications by systematists had established from five to twenty-two subdivisions of the species. He considered *P. pratensis* to be composed of two primary groups as based upon general morphological features.

TINNEY and AAMODT (14) concluded that the intensity of grazing and other ecological factors, including locality of origin, showed little effect upon the selection of particular morphological types. WELLHAUSEN and WEIBEL (15) reported that plants from populations in "pastures which had been maintained in a good stage of productivity over a long period of years" were generally more productive and much more resistant to leaf rust and mildew than comparable plants from pastures on areas of low fertility, particularly in relation to calcium and phosphorus. Plants from both types of pastures were compared under the same conditions.

ÅKERBERG (2) concluded that generally the form complexes of Eu-*pratensis* did not seem to be confined to any particular locality. The form complexes *irrigata*, *angustifolia*, and *alpigena*, however, were thought to occur in somewhat different habitats. The Eu-*pratensis* forms were observed to be highly variable and widely scattered, the others, including *alpigena*, being less so. Differences were not entirely clear, however.

Classification of progeny plants as to agreement in type with the parent form has been based upon relative morphologic similarity at any period of the growing season. MYERS (12) has pointed out that classification upon this basis may be uncertain, since many apparently normal plants produce progenies of varying character and may, therefore, be considered to be aberrant. Similar results were reported by SMITH and NIELSEN (13). BRITTINGHAM (4) has suggested that increased mortality in sexual types should also be considered in relation to variation. However, when certain obvi-

ous limitations of the criteria used are recognized, the percentage of aberrant (sexual) plants in a progeny has been generally accepted as an accurate index of the extent of sexual reproduction.

BRITTINGHAM (4) noted no significant differences to exist in percentages of aberrant frequency when comparing collections from different sources. Variability was somewhat lower, however, in many progenies of plants grown from commercial seed.

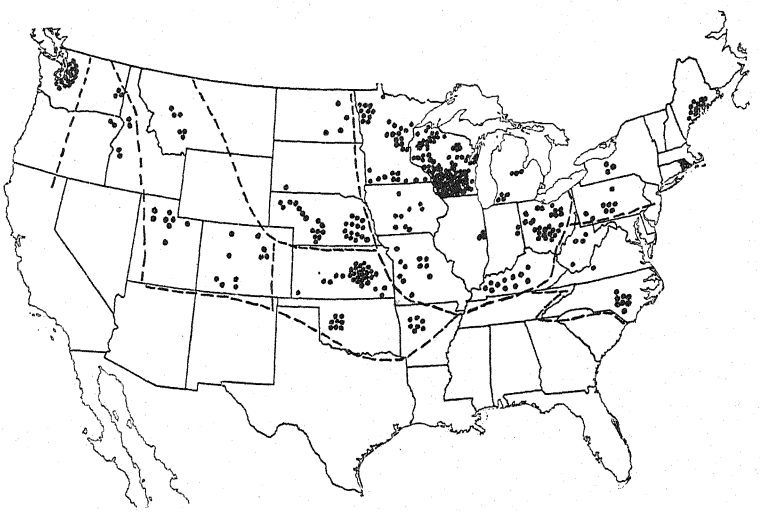


FIG. 1.—Distribution of 1941 collections of *Poa pratensis* in the United States. Dotted lines indicate general regional boundaries.

Material and methods

Progenies from a total of 800 collections from twenty-seven states were studied in the seasons 1941–44; these included 541 from Wisconsin. The distribution of the collections in the United States for 1941 is shown in figure 1 and that for Wisconsin in figure 2. The writers are indebted to a number of local investigators for many of the collections from other states. Unfortunately, most of the material from Illinois and Indiana was lost, this accounting for the lack of progenies from those states.

In making the collections no discrimination was shown as to location. Panicles were obtained from roadsides, fence rows, undergrazed and overgrazed pastures, and waste land. Some selection may have occurred, since only relatively well-developed panicles containing average or larger amounts of seeds were taken. Otherwise, relative culm height, texture, vigor, and other characters were ignored.

Progenies were grown from single panicles in a method similar to that described by TINNEY and AAMODT (14). Individual lots of seed were sown in steamed soil in small bread pans. Exceptions to the procedure were necessary when commercial lots, bulk strains, or foreign-plant introductions were included. Such strains have been propagated as composite lots and do not represent single plants. When seedlings were 2–3 inches tall, the sixteen to twenty-four

plants taken from each lot were re-established individually in small plant bands containing unsterilized soil. Subsequently the seedlings were transplanted into the field in early May, as progenies of the original panicles. Figures 3 and 4 illustrate the method of culture and the general appearance of progenies in early summer of the second year following establishment. Observations were made of the vigor and relative numbers of seedlings produced from each panicle, though these characters will not be considered in this presentation. The plants were usually vigorous, with percentage of survival very high each year. In the fields, progeny rows were 42 inches distant, and the plants were spaced 2 feet apart in the rows.

Observations were made upon relative vigor, uniformity, and disease reaction during late September of the first year. Plants not corresponding to the principal type of the progeny (aberrants) were noted, although first season's growth is not typical of that in later years. Detailed notes were taken periodically in the second season for a number of characters. These included uniformity, early (spring) vigor, early growth habit, aggressiveness, leafiness, leaf width, leaf length, foliage color, num-

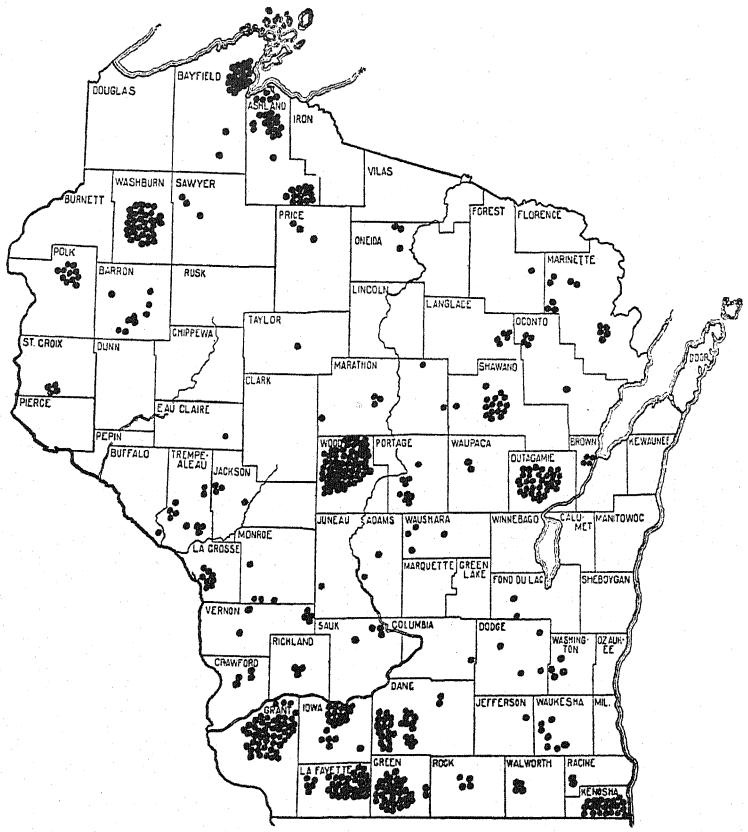
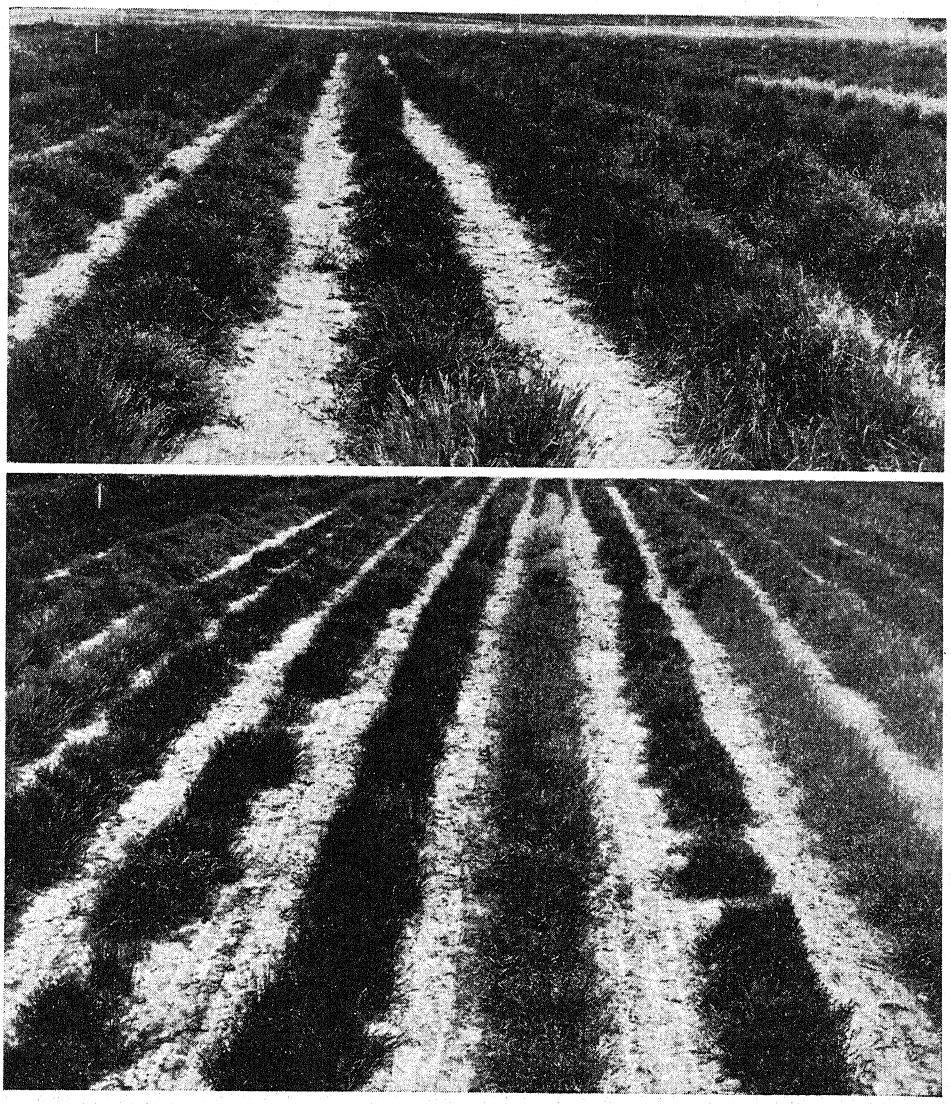


FIG. 2.—Distribution of 541 collections of *Poa pratensis* in Wisconsin, 1940–1943



FIGS. 3–4.—*Poa pratensis*. Fig. 3 (*above*), relatively vigorous progenies in rows early in second year of growth. Fig. 4 (*below*), progeny rows showing variations in color, vigor, and growth habit. Note highly sexual type on left. Consistent change of type in row indicates another progeny. Much of color difference is due to mildew infection.

ber and size of culms, plant height, earliness of seed maturity, reaction to diseases, and late (fall) vigor. Not all these characters will be considered in the present paper, though they were of use in determining the percentages of aberrant types. Data on early vigor were secured in 1943 only and represent only a part of the progenies.

The following tabulation summarizes the notes obtained concerning the morphological characters analyzed. Differences were indicated by the assignment of number values representing the relative development of these characters among plants and progenies. These number values are employed in several of the tables.

Early vigor. Lowest number most vigorous.
Growth habit. This character is based upon the general profile form of the plant and is somewhat related to aggressiveness: 1, prostrate; 2, near prostrate; 3, semiprostrate; 4, near-tussock; 5, tussock.
Aggressiveness. Lowest number most aggressive.
Leafiness. Lowest number most leafy.
Maturity. E, early; M, medium; L, late.
Late vigor. Lowest number most vigorous.

ALLISON and CHAMBERLAIN (3) reported the occurrence on bluegrass of the diseases studied, and the former author verified the identification of the causal organisms in subsequent years.

Results

ABERRANT FREQUENCY

UNITED STATES.—The breeding behavior of the 800 collections from different regions of the northern United States is shown in table 1. The large number of collections for the Central Lowland includes the Wisconsin lots later considered separately. The classification into physiographic regions is a modification of that of FENNEMAN (9).

TABLE 1

BREEDING BEHAVIOR OF POA PRATENSIS LOTS COLLECTED IN DIFFERENT REGIONS OF THE UNITED STATES WHEN GROWN AT MADISON, WISCONSIN

REGION	NO. OF COLLECTIONS	SEGREGATING		AV. % ABERRANTS BASED UPON—		MAX. % ABERRANTS
		No.	%	Total	Seg.	
Northeastern	35	31	88.6	26.2	29.6	60.0
Central Lowland	577	423	73.3	14.9	20.4	100.0
East Central	13	11	84.6	24.1	28.5	75.0
Northern Plains	35	24	68.6	11.7	17.1	56.0
Central Plains	45	39	86.7	17.0	19.6	44.0
Rocky Mountains	70	64	91.4	16.0	17.5	61.0
Northern Great Basin	9	7	77.7	9.1	11.7	31.0
North Pacific	16	7	43.8	14.7	33.6	38.0

Of the total number of collections, 606 (75.8%) produced segregating progenies. No attempt was made to subject the regional data to a rigid statistical analysis. The chi-square test indicated a close relation between the number of collections from each region and the number segregating, the average percentage reproducing apomictically being, therefore, reasonably similar in the several regions. The data for the North Pacific region may be an exception. Strains from the Rocky Mountain area were highest in the frequency of aberrants; those from

the Northeastern, Central Plains, and East Central sections were the most similar in this respect.

The average percentage of aberrant types for all regions was 16.5. Considering only those families developing aberrants, this percentage was 20.5. It should be stated, however, that these percentages are based upon certain morphological deviations in type, those classified as questionable not being included. The latter amounted to approximately 13.6% of the values given. It appears, therefore, that the percentages may be considered conservative. Significant differences appear to exist among the average percentages of aberrants in strains from the different regions. Progenies from the North Pacific, Northeastern, and East Central areas were generally higher in frequency of aberrants. Although such differences may be real, the chi-square test indicated that variances for the several regions were not homogeneous and that the significance of differences could not be determined on a general basis. Wide ranges in the maximum percentage of aberrant types are indicated, with larger groups tending to exhibit highest values, as might be expected.

TABLE 2

BREEDING BEHAVIOR OF POA PRATENSIS LOTS COLLECTED FROM DIFFERENT SOIL TYPES IN WISCONSIN

SOIL AREAS	NO. OF COLLECTIONS	SEGREGATING		AV. % ABERRANTS BASED UPON—		MAX. % ABERRANTS
		No.	%	Total	Seg.	
Knox* (silt loams and loams)	131	85	65	12	18	100
Miami and Carrington† (silt loams and loams)	97	72	74	18	24	100
Miami and Carrington‡ (sands and sandy loams)	13	9	69	19	13	63
Superior§ (clays and clay loams; northern area)	39	26	67	11	17	75
Superior‖ (clays and clay loams; southern area)	37	30	81	15	18	52
Sands¶	102	68	67	10	15	57
Spencer	41	27	66	12	18	67
Kennan** (silt loams and loams)	56	40	71	12	16	67
Kennan†† (sands and sandy loams)	12	10	83	13	16	50
Peat	5	1	20	5	6	25
Wet land	8	4	50	1	30	6

* Unglaciated region of southwestern Wisconsin characterized by occurrence of Fayette, Dubuque, Gale, Tama, and Dodgeville series.

† Glaciated region of eastern Wisconsin; Carrington includes Parr series principally but with some Carrington.

‡ Glaciated area of northeastern Wisconsin; characterized by occurrence of Onaway, Trenary, and Kewaunee series.

§ Ontonagon series.

‖ Superior and Kewaunee series.

¶ Coloma, Plainfield, and Boone series.

** Includes Kennan, Milaca, Cloquet, and Iron River series.

†† Includes Vilas, Omega, and Hiawatha series.

WISCONSIN.—An analysis is given in table 2 of the percentages of collections producing aberrant types and of the average percentages of aberrants for different soil-type groups in Wisconsin. The classification of soil areas is general and follows that of WHITSON (16). This classification is broad and regional rather than specific, as the individual soil types of the sites at which panicles were collected were not identified. The chi-square test resulted in a very high p value, indicating that relative numbers of progenies segregating were similar for the various

soil areas. The data suggest that collections from peat soil and other wet land are less likely to produce aberrant types than are those from other soil areas, although the number of samples is very small. It is problematical whether differences occurring among collections from other soil areas are significant. The highest percentage of segregating collections is shown for Kennan sands and sandy loams; the next highest is for Superior clays and clay loam (southern area).

A chi-square test of the variances for the percentages of segregation within progenies from the several soil classes showed that the error was heterogeneous. Removal of several selected groups from the analysis did not serve to reduce the chi-square value. The highest average percentage of aberrant plants occurred in collections from wet land; the next highest was obtained for Miami and Carrington silt loam and loam regions, where an average of 24% aberrants based upon the segregating collections was obtained. Although there is some variation in the data for the several soil areas, no obvious relationships between soil and percentage of segregation are apparent. A wide range exists in maximum percentage of aberrants among soils of different classes. Maxima tend to be higher where larger numbers of collections were available, but the data for sandy soils are somewhat divergent from this tendency. It would seem that the ultimate maxima for any soil areas might be similar but that the frequency of such maxima might, for various reasons, be considerably different.

Table 3 contains data with the collections classified according to the length of the growing season where they were obtained. The divisions follow those given in *Climate and man* (7). The chi-square test indicated a very good fit of the classes segregating in proportion to their total numbers. With the exception of the class in the growing season of 90–100 days, they are essentially similar in the percentages of the lots segregating. In this particular class the number of collections was very small, and the percentage is, therefore, relatively unreliable.

Wide differences are shown among the percentages of aberrants based upon the total number of collections. The 90–100-

TABLE 3

BREEDING BEHAVIOR OF POA PRATENSIS LOTS COLLECTED IN WISCONSIN AND CLASSIFIED ACCORDING TO LENGTH OF GROWING SEASON

CLASS	AV. GROWING SEASON (DAYS)	NO. OF COLLECTIONS	SEGREGATING		AV. % ABERRANTS BASED UPON—		MAX. % ABERRANTS
			No.	%	Total	Seg.	
1	90–100	3	3	100	39	39	53
2	100–110	14	10	71	8	11	50
3	110–120	61	42	69	9	13	77
4	120–130	104	68	65	11	16	75
5	130–140	47	31	66	9	14	50
6	140–150	137	97	71	16	23	80
7	150–160	120	81	68	12	18	100
8	160–170	36	25	69	14	20	56
9	170–180	22	16	73	17	23	100

day class is again the most divergent, though numbers are too small for significance. With this exception, however, the strains derived from areas with longer growing seasons seemingly have somewhat higher average percentages of aberrants. This relationship is less obvious when the percentages of aberrants among segregating types are considered. The test for homogeneity showed the variance to be heterogeneous in this group. Exclusion of classes 1 and 9, however, reduced the chi-square value to an insignificant amount. The F values in the subsequent analysis of variance indicated that significant differences existed between certain percentages. Calculation of the least significant differences for the numerous possible paired comparisons showed 140–150-day lots to be significantly higher in average percentage of aberrants than the 100–110-, 110–120-, 120–130-, 130–140-, and 150–160-day lots. In general, the groups from areas of longer growing season appear to have higher percentages of plants reproducing sexually. The shortest season class (90–100 days) is an exception, but the number of progenies in this group is very small. Relationships among different regions for maximum percentages of aberrants indicate that the maximum is higher, as expected, for the classes with the larger numbers of collections, though some exceptions occur.

Data based upon the classification of collections according to geographic areas of Wisconsin are given in table 4. These areas are from the differentiations made by MARTIN (10). In general, a close similarity is indicated among different regions with respect to the percentages of the collections segregating. Since the chi-square test indicated variance to be homogeneous, an analysis of variance was made.

TABLE 4

BREEDING BEHAVIOR OF POA PRATENSIS LOTS COLLECTED IN WISCONSIN AND CLASSIFIED ACCORDING TO GEOGRAPHIC AREAS

GEOGRAPHIC AREA	NO. OF COLLECTIONS	SEGREGATING		AV. % ABERRANTS BASED UPON—		MAX. % ABERRANTS
		No.	%	Total	Seg.	
Eastern Ridge and Lowlands	103	79	77	16	21	100
Central Plains	103	70	68	11	15	63
Western Upland	192	128	67	14	21	100
Northern Highland	107	73	68	11	16	100
Lake Superior Lowland	42	28	67	11	17	75

P exceeds 0.05; variance between regions, 719.925.

The significantly different paired comparisons for the frequency of aberrants indicate that the Western Upland exceeds in frequency of aberrants the Central Plains and Northern Highland areas and that the Eastern Ridge and Lowlands similarly exceeds the Northern Highland region. The data show that the Eastern Ridge and Lowlands and the Western Upland areas average higher than other geographical regions in percentages of aberrants. This suggests that collections from these areas give progenies somewhat higher in percentages of aberrants based upon the total number and also upon the number of segregating

TABLE 5

NUMERICAL FREQUENCIES OF PERCENTAGE CLASSES OF ABERRANT PLANTS IN STRAINS OF POA PRATENSIS BASED UPON SOIL AREA, LENGTH OF GROWING SEASON, AND GEOGRAPHIC AREA WHERE COLLECTED IN WISCONSIN

Classification	Classes for percentage of aberrant plants											
	0	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	Total
	Soil areas*											
Knox (silt loams and loams)	46	33	27	12	3	7	....	....	....	....	2	131
Miami and Carrington (silt loams and loams)	25	25	15	9	10	8	....	2	2	....	1	97
Miami and Carrington (sands and sandy loams)	4	2	4	....	1	....	1	1	....	....	....	13
Superior (clays and clay loams; northern area)	13	9	10	3	3	....	....	....	1	....	....	39
Superior (clays and clay loams; southern area)	7	10	11	4	3	1	1	....	....	....	....	37
Sands	34	29	28	3	1	3	4	....	....	....	....	102
Spencer	14	11	6	6	3	....	....	1	....	....	....	41
Kennan (silt loams and loams)	16	19	12	4	1	2	1	1	....	....	....	56
Kennan (sands and sandy loams)	2	4	4	1	....	1	....	....	....	....	....	12
Peat	4	1	....	....	....	....	....	....	....	....	....	5
Wet land	4	1	1	1	....	....	....	....	1	....	....	8
Total	169	144	118	43	25	22	7	5	4	....	3	540
	Length of growing season											
90–100 days	....	....	1	....	....	....	2	....	....	....	....	3
100–110 days	4	8	1	....	....	1	....	....	....	....	....	14
110–120 days	19	20	19	1	....	1	....	....	1	....	....	61
120–130 days	36	28	23	10	4	....	1	1	1	....	....	104
130–140 days	16	16	10	2	1	2	....	....	....	....	....	47
140–150 days	40	30	25	14	9	13	3	2	1	....	....	137
150–160 days	39	33	27	10	3	3	2	1	1	....	1	120
160–170 days	11	5	11	3	4	1	1	....	....	....	....	36
170–180 days	6	6	3	3	3	....	....	....	....	....	1	22
Total	171	146	120	43	24	21	9	4	4	....	2	544
	Geographic areas											
Eastern Ridge and Lowlands	23	31	22	11	10	3	2	....	1	....	1	104
Central Plains	29	33	27	7	0	4	1	2	....	....	....	103
Western Upland	65	40	39	18	9	14	3	2	1	....	1	192
Northern Highland	35	35	22	7	3	1	2	....	1	....	1	107
Lake Superior Lowland	14	10	13	1	3	....	....	....	1	....	....	42
Total	166	149	123	44	25	22	8	4	4	....	3	548

* Cf. table 2.

collections than do those from other regions. Maximum percentages of aberrants appear to be reached, as previously indicated, when larger numbers of collections are involved, although the Central Plains area is an exception.

The relative frequencies of percentage classes of aberrant plants for the different soil groups of Wisconsin (16) are shown in table 5. The low and zero percentage classes contain most of the collections for all soil areas. There appears to be some tendency for collections from Miami and Carrington silt loam and loam soils to range into somewhat higher classes. Lots from Knox and sandy soils showed somewhat higher frequencies than average in the lower percentage classes. A less marked trend is indicated for collections from certain other soil types to show irregular tendencies. This is particularly notable for lots from Miami and Carrington and Kennan soils.

The frequencies of percentage classes of aberrant plants based upon classification for length of the growing season are also given in table 5. There is an apparent tendency for lots collected from regions having 120–130-, 140–150-, and 150–160-day growing seasons to have somewhat higher frequencies of aberrants than the average. The general relation of the length of the growing season to the frequency of aberrants, however, indicates no particularly striking tendencies.

Data for classification based upon geographic areas in table 5 suggest that lots from the Eastern Ridge and Lowlands and Western Upland areas have somewhat more widely distributed frequencies than the average in the various classes of aberrant plant percentage; the Western Upland area had a somewhat higher proportion in the zero class. Other groups are generally similar to one another in relative distribution.

Growth characters

United States.—The numerical frequencies shown in table 6 are of the United States collections classified for early and late vigor, growth habit, aggressiveness, leafiness, and maturity. Graphical examinations have been made of the frequency distributions of the percentages in the various numerical classes considering each region for each character. These studies indicated that the distributions are essentially similar among all regions for each trait observed. This is particularly true for early vigor, growth habit, and aggressiveness. Comparisons of the relationships among regions for leafiness, maturity, and late vigor were of greater contrast. Only character relationships which were somewhat variable among regions will be discussed.

There was some indication that strains from the Northern Great Basin were more often of the low prostrate growth habit than were those from other regions. The number of samples for this region, however, is quite small. Collections from the East Central region showed a slightly different distribution for aggressiveness than those from other regions, the former being generally less aggressive. Collections from the Northeastern and North Pacific regions were somewhat exceptional to the general average of the material from other regions for leafiness characters; much higher percentages of these two groups were leafy, with the stemmy types being relatively infrequent. In relative maturity the lots from the Northern Plains and the Northern Great Basin were generally earlier than those of other regions from which most collections were classified as medium late to late. Differences in this respect were quite marked. The most diversity occurred with respect to the degree of vigor shown by collections from different

regions at the time of maturity. Material from the Northern Plains and from the Central Plains appeared to be generally more vigorous than that from other regions. Very few lots were classified as extremely low in late vigor.

The numbers of collections from the several regions are quite different, and the relative representation is not entirely comparable. It is concluded, however, that there appears to be little characteristic regional diversity among strains for early vigor, growth habit, and aggressiveness but that there is some contrast among regional lots in leafiness, relative maturity, and late vigor. Whether such

TABLE 6

NUMERICAL FREQUENCIES OF STRAINS OF POA PRATENSIS FROM VARIOUS REGIONS AND STATES IN CLASSES FOR VIGOR, GROWTH HABIT, AGGRESSIVENESS, LEAFINESS, AND MATURITY

Region and state	Early vigor				Growth habit					Aggressiveness			Leafiness			Maturity			Late vigor			
	1	2	3	4	1	2	3	4	5	1	2	3	1	2	3	E	M	L	1	2	3	4
1. Northeastern:																						
Maine	...	5	5	...	...	...	4	3	3	...	7	3	5	2	3	3	1	6	1	3	4	2
New York	1	2	...	...	...	...	2	...	1	1	1	1	1	2	...	...	1	2	1	1	1	...
Pennsylvania	...	5	3	...	...	...	5	3	...	1	4	3	5	3	...	...	...	8	2	4	2	...
Rhode Island	1	6	7	...	...	2	6	5	1	1	12	...	12	1	1	...	1	11	5	5	3	1
Total	2	18	15	...	...	2	17	11	5	3	24	7	23	8	4	3	3	27	9	13	10	3
2. Central Lowland:																						
Illinois	1	1	1	...	...	1	1	1	...	1	2	...	1	2	...	...	...	3	3	...	...	...
Indiana	1	1	...	...	...	...	1	1	...	1	1	...	2	...	...	...	2	...	2	...	...	...
Iowa	...	5	4	...	1	3	3	2	...	...	5	4	2	6	1	...	2	7	4	2	3	...
Kentucky	2	8	1	...	...	2	5	3	1	1	9	1	4	6	1	...	3	8	6	4	...	...
Michigan	1	6	3	...	...	...	5	4	1	3	5	2	5	4	...	...	1	9	5	3	2	...
Minnesota	6	20	10	...	...	15	11	7	3	4	28	4	16	18	2	...	6	19	19	14	3	...
Missouri	3	7	1	...	2	2	6	...	1	1	9	1	6	5	...	...	4	7	4	6	1	...
Ohio	10	11	5	...	1	3	12	8	2	8	14	3	17	8	1	...	6	20	14	9	2	...
Wisconsin	20	65	41	...	10	30	55	19	11	18	69	39	36	70	20	3	54	69	43	53	23	6
Total	44	124	66	...	14	56	99	45	19	37	142	54	89	119	25	3	78	142	100	91	34	6
3. East Central:																						
North Carolina	...	6	2	...	...	2	3	2	1	...	1	7	2	6	...	...	5	3	5	3	...	...
Virginia	...	...	1	...	...	...	1	...	...	...	1	...	1	...	...	...	...	1	1	...	...	...
West Virginia	2	...	2	...	...	...	1	1	2	1	2	1	1	3	...	...	2	2	3	1	...	...
Total	2	6	5	...	...	2	5	3	3	1	4	8	4	9	...	...	7	6	9	4	...	...
4. Northern Plains:																						
Nebraska	7	20	3	...	5	7	11	4	3	8	21	1	5	20	5	...	20	10	21	9	...	...
North Dakota	1	3	...	...	...	1	3	...	...	...	3	...	1	2	...	...	2	2	2	1	1	...
South Dakota	...	1	...	...	...	...	...	...	1	...	1	...	1	...	...	...	1	...	1	...	...	...
Total	8	24	3	...	5	8	14	4	4	8	25	1	7	22	5	...	23	12	24	10	1	...
5. Central Plains:																						
Arkansas	1	5	1	...	1	...	3	1	2	1	4	2	3	3	1	...	3	4	2	5	...	...
Kansas	4	17	9	...	...	6	19	4	1	1	26	3	7	20	3	...	6	24	12	12	5	1
Oklahoma	4	3	1	...	...	2	1	3	2	...	7	1	6	2	...	...	2	6	1	3	3	1
Total	9	25	11	...	1	8	23	8	5	2	37	6	16	25	4	...	11	34	15	20	8	2
6. Rocky Mountains:																						
Colorado	2	8	...	...	...	2	5	2	1	...	8	2	5	5	...	...	4	6	8	1	1	...
Montana	1	3	2	...	1	1	3	...	1	2	3	1	...	6	...	...	5	1	5	1	...	...
Utah	...	10	...	...	1	4	4	1	...	6	4	...	3	2	5	1	4	5	4	2	...	...
Total	3	21	2	...	2	7	12	3	2	8	15	3	8	13	5	1	13	12	17	4	1	...
7. Northern Great Basin:																						
Idaho	...	3	1	...	...	3	1	...	...	1	2	1	...	3	1	...	3	1	3	1	...	...
Oregon	...	1	1	...	...	1	1	...	...	...	2	...	...	2	...	...	2	...	1	1	...	...
Washington	...	1	2	...	...	...	1	2	...	...	1	2	...	3	...	2	1	...	1	2	...	...
Total	...	5	4	...	...	4	3	2	...	1	5	3	...	8	1	2	6	1	5	4	...	...
8. North Pacific:																						
Washington	2	7	7	...	3	2	9	1	1	7	7	2	9	7	...	...	4	11	2	9	5	...

differences are significant with respect to the natural development of regional types of bluegrass appears to be questionable.

WISCONSIN.—Numerical frequencies are presented in table 7 for the soil, growing season, and geographic classifications of the Wisconsin collections for the several characters of growth. It is somewhat difficult to make comparisons because of insufficient numbers in some instances. These frequencies are not directly comparable with those for the collections for the entire United States. Much diversity was shown with respect to soil areas.

Considerable diversity in frequencies for growth habit existed among the col-

TABLE 7

NUMERICAL FREQUENCIES OF STRAINS OF POA PRATENSIS IN CLASSES FOR VIGOR, GROWTH HABIT, AGGRESSIVENESS, LEAFINESS, AND MATURITY WHEN BASED UPON SOIL AREA, LENGTH OF GROWING SEASON, AND GEOGRAPHIC AREA WHERE COLLECTED IN WISCONSIN

CLASSIFICATION	EARLY VIGOR			GROWTH HABIT					AGGRESSIVENESS				LEAFINESS				MATURITY			LATE VIGOR				
	1	2	3	1	2	3	4	5	1	2	3	4	1	2	3	4	E	M	L	1	2	3	4	5
Soil areas*																								
Knox (silt loams and loams)	8	19	14	14	39	35	12	3	14	36	23	32	25	61	18	0	26	45	34	14	41	35	12	3
Miami and Carrington (silt loams and loams)	3	6	8	4	21	20	15	1	9	16	24	10	23	25	11	1	21	22	18	6	11	17	23	3
Miami and Carrington (sands and sandy loams)	1	8	3	1	2	4	3	2	2	6	5	0	2	9	3	0	1	5	7	0	3	4	4	2
Superior (clays and clay loams; northern area)	0	3	2	6	7	7	14	5	9	14	4	13	15	17	5	2	23	7	9	1	3	8	20	7
Superior (clays and clay loams; southern area)	0	0	0	2	2	0	0	0	0	1	0	3	0	1	3	0	2	2	0	0	1	0	2	1
Sands	7	15	6	11	28	37	9	5	11	35	26	23	17	42	16	15	42	36	12	6	21	29	31	5
Spencer	1	4	2	3	7	13	16	2	9	6	15	11	24	12	2	3	18	9	15	3	7	13	16	2
Kennan (silt loams and loams)	1	0	0	1	8	18	7	1	3	2	16	14	10	16	6	3	19	12	4	1	2	13	16	3
Kennan (sands and sandy loams)	0	4	1	0	5	2	0	2	0	5	3	1	1	5	3	0	3	5	1	0	5	2	0	2
Peat	0	0	0	0	0	3	1	1	0	2	3	0	1	2	1	1	5	0	0	0	0	3	1	1
Wet land	1	2	0	0	1	3	2	1	1	4	1	1	2	7	4	1	5	0	3	1	1	3	2	1
Total	22	61	36	42	120	142	79	23	58	127	120	108	120	197	72	26	165	143	103	32	95	127	127	30
Length of growing season																								
90–100 days	0	0	0	0	1	2	0	0	0	2	0	1	0	2	1	0	1	1	1	0	2	0	1	0
100–110 days	0	0	0	0	2	8	3	1	1	1	5	7	6	7	1	0	8	4	2	1	0	6	5	2
110–120 days	3	4	1	13	22	17	5	3	3	15	26	17	12	29	12	8	18	27	16	5	7	15	31	3
120–130 days	0	6	4	7	20	30	34	7	17	26	25	29	37	33	18	8	25	40	31	17	19	30	27	4
130–140 days	3	6	6	3	13	20	9	3	5	15	18	10	12	18	12	6	8	27	13	6	15	16	11	0
140–150 days	5	18	9	4	7	32	14	5	12	22	25	3	21	28	9	4	25	10	18	11	21	20	8	2
150–160 days	7	15	6	12	36	27	7	3	13	28	18	29	15	53	15	0	27	32	26	13	15	16	39	2
160–170 days	4	13	9	4	11	8	4	3	3	17	6	4	8	18	4	0	2	10	18	7	11	5	4	1
170–180 days	1	1	3	1	7	2	4	0	1	2	10	1	4	6	3	0	6	2	6	2	1	3	8	0
Total	23	63	38	44	119	146	80	25	55	128	133	101	115	194	75	26	120	162	131	62	91	111	134	14
Geographic areas																								
Eastern Ridge and Lowlands	4	16	4	5	25	21	16	2	12	23	25	15	25	30	15	0	18	21	29	13	18	16	27	4
Central Plains	7	8	7	11	23	40	12	7	6	19	40	28	22	47	22	13	21	50	22	10	18	30	35	...
Western Upland	8	31	23	14	43	39	13	4	15	50	24	24	24	73	15	1	21	39	53	18	39	22	32	1
Northern Highland	2	5	2	9	21	36	26	7	14	23	29	34	34	46	20	10	32	37	31	7	19	32	35	7
Lake Superior Lowland	0	5	2	6	7	11	14	4	8	15	6	13	13	20	7	2	9	19	14	15	6	11	8	2
Total	21	65	38	45	119	147	81	24	55	130	124	114	118	216	79	26	101	166	149	63	90	111	137	14

* Cf. table 2.

lections from the various soil classes, but few of these appeared to have any notable characteristics. The classification for aggressiveness showed considerable variation among lots. Those from Spencer and Kennan silt loam and loam areas generally had somewhat similar frequencies; those from the two Superior clay and clay loam areas had similar distributions, with a tendency toward nonaggressive types. Other than these generalizations, the relationships were sufficiently irregular as to appear to be of little significance.

In the frequency distribution of collections classified for leafiness, leafy types were relatively more numerous in the data for the Spencer soil area than for any of the other soil groups; otherwise the frequency distributions for leafiness classes were quite similar.

Frequencies for maturity classes showed greater divergence among collections from different soil areas than did those for other characters. Lots from peat soil were notably early in maturity. Those from Kennan silt loams and loams, Kennan sands and sandy loams, wet land, and Superior clays and clay loams were also relatively early in maturity.

Types from Kennan sand and sandy loam soils had a high frequency of vigorous strains in the classification for late vigor. This is of doubtful significance. A relatively high frequency of marked vigor was also shown by lots from wet land. A relatively high frequency of moderate to poor late vigor was indicated for types derived from Superior clays and clay loams. Lots from Spencer soil and Kennan silt loam and loam soils showed somewhat similar distributions but had relatively higher frequencies in the medium vigorous and vigorous classes.

Frequency comparisons for classification based upon length of growing season (table 7) show that the relationships among classes for early vigor, growth habit, and leafiness are fairly comparable, no marked differences in regions being indicated. The collections from areas of shorter growing seasons showed relatively high percentages of intermediate types with respect to growth habit, whereas the 160–170- and 170–180-day regions presented a somewhat disproportionate frequency of low-growing types. Collections from the 140–150-day region tended to resemble those from the areas of shorter growing seasons.

Distributions for the seasonal areas based upon aggressiveness were quite irregular. Relatively more aggressive types were found in the 90–100-day region; a fairly high percentage of aggressive types was also shown in the 160–170-day region. The frequency of such types was low in the 170–180-day region, and the 100–110-day region showed an unexpectedly high proportion of nonaggressive types. Distributions of frequencies in leafiness classes for the various lengths of growing seasons were very similar, no notable tendency being indicated.

Classification for maturity presented the most irregular data of any of the characters studied, no relationship appearing to exist among regions for this character. There was a tendency for lots from the 100–110-day region to be early in maturity. Collections from the 110–120-day area showed no apparent relationships. Lots from the 160–170-day area were relatively late in maturity, but those from the 170–180-day area showed no apparent relationship to the groups from the areas of shorter season.

The distributions of frequencies in the classification for late vigor were generally quite different for the various seasonal groups. Lots from the 90–100-day group showed a relatively high percentage of

progenies of marked vigor, but those from the 100–110- and 110–120-day groups exhibited a general tendency to lack vigor, as did those from the 150–160 and 170–180 groups. Data for the 120–130, 130–140, and 140–150 groups present fairly comparable distributions, with some tendency for types to be average in vigor. The 160–170-day class was higher than average in vigor. It should be noted in the classifications for growing season that only three collections were represented in the 90–100-day class.

In summary it may be said that grouping the collections on the basis of the average lengths of the frost-free season indicates much variation among groups with respect to the characters studied but no consistent relationships. Most diversity exists in the characters of aggressiveness, maturity, and late vigor, and it is very difficult to see any general trends.

When the data were grouped by geographic origin of collections (table 7), the frequency distributions among the early vigor classes were very similar for the several areas except for the Central Plains region, where fewer intermediate types occurred. In growth habit the Eastern Ridge and Lowlands and the Western Upland areas showed proportionately higher numbers of low-growing types, while the Lake Superior Lowland group had a proportionately higher frequency of the bunch type. Considerable diversity was shown among regions in the classes for aggressiveness; only the Western Upland and Lake Superior Lowland distributions were closely similar. The latter area showed a proportionately large number of nonaggressive types, as did the Northern Highland group. The Central Plains class was relatively high in intermediate types. Distributions for frequencies of classes for leafiness were very similar among the regions with the Western Upland group containing more intermediate types. In the maturity classification the Eastern Ridge and Lowlands and the Western Upland classes were similar in their relative frequencies. The other three areas were somewhat alike among themselves, but the Northern Highland group contained proportionately fewer intermediates. Classification for late vigor showed much irregularity in relation to geographic areas. The Eastern Ridge and Lowlands, the Central Plains, and the Northern Highland groups were not widely divergent. Lots from the Western Upland and Lake Superior Lowland regions were divergent and between themselves showed somewhat opposite frequencies except for the least vigorous classes.

Among the several characters analyzed the variation in leafiness was most comparable, whether groups were based upon soil areas, upon relative lengths of the growing season, or upon geographic sections in Wisconsin. Early vigor and growth habit might be considered next most constant, whereas aggressiveness and late vigor were most variable.

Classification for soil areas produced most irregularity in relative frequencies, while grouping based upon geographic areas showed least diversity.

Plant height

United States.—Although plant height at maturity is perhaps a character of little practical importance, it seemed evident that its value might be more accurately measured than other plant features. A comparison of strains upon this basis for the geographic regions of the United States is presented in table 8, although the number of strains was generally insufficient to permit detailed comparisons of frequencies in the differ-

ent regions. The collections from the Northeastern and the Central Lowland sections of the country perhaps include types averaging somewhat shorter in culm length. Distribution of the lots from the Central Lowland also suggests a negative skewness compared with the average frequencies of all progenies. It would appear very doubtful whether the differences indicated are of any significance.

WISCONSIN.—Collections from the various soil areas of Wisconsin have been classified for culm height (table 9). Insufficient information is available to make comparisons between some regions, and data are meager for all soil regions. Comparing relative frequencies, distributions are somewhat similar. Progenies from plants of Spencer soils and of Kennan silt loams and loams were somewhat deficient in types of average height and were more variable than the average.

Results of the classification based on the length of the growing season are also presented in table 9. The 100–110- and 130–140-day classes were more variable than the other groups with proportionately more short and more tall types. Other distributions approached the average type. Strains from the 150–160-day area included an exceptional number of medium short types; a two-peaked curve resulted.

Data for geographic areas (table 9) suggest that all distributions resemble the normal, though minor variations occur. Western Upland and Northern Highland strains included proportionately more medium short types, while the Eastern Ridge and Lowlands and Northern Highland areas had relatively numerous tall biotypes.

TABLE 8

NUMERICAL FREQUENCIES FOR HEIGHT OF PLANT OF STRAINS OF POA PRATENSIS FROM GEOGRAPHIC REGIONS OF THE UNITED STATES

REGION	PLANT HEIGHT IN INCHES AT MATURITY																			
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Total
Northeastern	1	1	1	...	3	3	4	4	3	8	2	1	1	1	1	...	1	...	...	...	35
Central Lowland	...	1	2	6	3	3	7	18	17	26	38	38	21	19	10	7	4	11	1	3	235
East Central	...	...	...	...	...	...	...	...	2	2	...	2	2	4	1	...	...	...	...	...	13
Northern Plains	...	...	...	...	...	3	...	2	1	2	3	6	7	7	1	2	...	...	...	...	34
Central Plains	...	...	...	...	1	3	3	3	5	9	4	5	4	5	2	...	1	...	...	...	45
Rocky Mountains	...	...	...	...	...	...	3	...	1	1	2	6	2	6	2	1	1	1	...	...	26
Northern Great Basin	...	...	...	...	...	...	1	...	1	...	...	2	2	2	...	1	...	...	...	...	9
North Pacific	1	...	...	...	1	1	...	2	...	...	3	2	2	1	1	2	...	...	...	...	16
Total	2	2	3	6	8	13	18	29	30	48	52	62	41	45	18	13	7	12	1	3	413

Groupings based upon length of the growing season appeared to show more diversity in plant height than those based upon soil and geographic areas.

DISEASE REACTIONS

Certain diseases of Kentucky bluegrass occurred with sufficient intensity and general distribution to permit observation of strain reactions. The diseases were not equally abundant in all seasons, and plant reactions were somewhat variable from year to year. The diseases observed included leaf rust caused by *Puccinia poae-sudeticae* (Westend.) Jørstad and *P. rubigovera* (DC.) Wint.;

TABLE 9

NUMERICAL FREQUENCIES OF STRAINS OF POA PRATENSIS IN CLASSES FOR HEIGHT OF PLANT WHEN BASED UPON SOIL AREA, LENGTH OF GROWING SEASON, AND GEOGRAPHIC AREA WHERE COLLECTED IN WISCONSIN

Classification	Culm height in inches at maturity																			
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Total
Soil areas*																				
Knox (silt loams and loams)	...	1	2	1	4	5	3	14	7	14	16	12	11	5	7	1	2	...	...	105
Miami and Carrington (silt loams and loams)	...	...	...	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	2
Miami and Carrington (sands and sandy loams)	...	1	4	...	2	...	1	3	8	11	6	5	6	6	2	1	...	...	...	56
Superior (clays and clay loams; northern area)	...	...	...	1	...	...	2	1	1	1	1	2	...	...	1	1	2	...	...	13
Superior (clays and clay loams; southern area)	...	1	...	...	...	...	1	1	1	3	3	3	...	2	...	...	1	...	...	16
Sands	...	...	...	...	...	...	1	...	...	...	...	...	2	1	...	...	...	...	...	4
Spencer	1	...	1	...	2	...	3	4	8	9	4	4	5	9	3	4	2	...	1	60
Kennan (silt loams and loams)	...	...	...	...	2	2	1	5	4	2	5	3	2	1	3	3	...	...	...	33
Kennan (sands and sandy loams)	...	...	3	1	2	2	4	4	3	2	...	...	1	...	1	...	1	...	1	25
Peat	...	...	2	...	...	...	...	...	...	1	2	1	2	...	...	1	...	...	...	9
Wet land	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	1	...	...	3
Total	1	3	12	3	12	10	17	33	32	43	38	30	29	24	17	11	9	...	2	326
Length of growing season																				
90–100 days	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
100–110 days	...	...	2	1	1	2	2	2	...	1	...	...	1	...	1	...	...	...	...	13
110–120 days	1	...	1	...	2	...	3	2	6	7	4	5	1	5	2	1	2	...	1	43
120–130 days	...	1	...	1	3	2	1	8	6	7	7	3	4	3	6	4	1	...	...	57
130–140 days	...	...	2	...	1	2	2	3	7	1	2	2	2	1	...	2	2	...	...	29
140–150 days	...	1	2	...	2	1	2	2	7	11	5	3	7	4	1	1	1	...	1	51
150–160 days	...	1	...	1	3	2	6	11	2	7	16	10	10	8	4	5	4	...	...	90
160–170 days	...	...	1	...	...	...	1	2	2	7	4	5	4	3	1	...	...	...	...	30
170–180 days	...	...	4	...	...	...	...	1	1	2	1	2	...	...	2	...	...	...	...	13
Total	1	3	12	3	12	9	17	31	32	43	39	30	29	24	17	13	10	...	2	327
Geographic areas																				
Eastern Ridge and Lowlands	...	1	4	...	1	1	5	3	9	11	7	6	7	9	3	4	3	...	...	74
Central Plains	1	...	3	1	3	1	3	6	8	7	5	5	3	4	3	2	2	...	...	57
Western Upland	...	1	2	...	4	4	4	12	7	15	17	14	13	6	5	1	3	...	1	109
Northern Highland	...	...	2	2	5	3	4	10	5	7	6	3	5	3	7	5	1	...	1	69
Lake Superior Lowland	...	1	1	...	...	...	1	2	2	3	3	3	1	2	...	...	1	...	...	20
Total	1	3	12	3	13	9	17	33	31	43	38	31	29	24	18	12	10	...	2	329

* Cf. table 2.

powdery mildew (*Erysiphe graminis* DC.), and leaf spot (*Helminthosporium vagans* Drechs.). It is thought, however, that *P. rubigovera* was relatively unimportant in the season when leaf-rust reaction was observed. Since data were not obtained for all collections, numbers may not coincide with those for other characters. The plant reactions were recorded numerically; 0, no disease observed; 1, resistant; higher numbers represented progressively a more susceptible reaction. Those classified as of reaction 3 were considered to be very susceptible.

TABLE 10

NUMERICAL FREQUENCIES FOR DISEASE-REACTION CLASSES OF STRAINS OF POA PRATENSIS FROM VARIOUS REGIONS OF THE UNITED STATES

Region	Mildew				Leaf rust			
	0	1	2	3	0	1	2	3
Northeastern	17	3	6	9	13	19	2	1
Central Lowland	41	22	28	17	9	76	17	7
East Central	3	2	5	3	2	10	1	0
Northern Great Plains	4	19	11	0	1	27	4	2
Central Great Plains	18	7	16	4	8	28	8	2
Rocky Mountains	8	8	10	0	3	16	6	3
Northern Great Basin	2	5	3	0	1	5	2	1
North Pacific	4	5	1	2	4	9	0	0
Total	97	71	80	35	41	190	40	16

UNITED STATES.—Numerical frequencies of reaction types for the several regions of the United States are shown in table 10. Insufficient leaf spot developed in 1943 to provide information concerning reaction toward this disease. The frequency distributions for classes of reaction to mildew were very diverse for the several regions; those for the Northern Plains and for the Northern Great Basin were essentially similar, and those for the Rocky Mountain and North Pacific areas varied in a different manner. Other regional distributions comprised a third group. The Northeastern region showed a somewhat higher proportion of mildew-free strains but also a higher number of susceptible ones. Intermediate reaction types were lacking. The highest proportion of susceptible types occurred in the Northern Plains group. Approximately 34% of all collections studied were classified as mildew-free, whereas 12% were very susceptible.

The frequency distributions for leaf-rust classification were very closely similar for all regions. High percentages of the collections were rust-free or were slightly infected, but no regional tendencies were evident.

WISCONSIN.—Data for classification according to soil in Wisconsin are given in table 11. Frequency distributions for mildew reaction were generally similar among the soil groups except those for Kennan sands and sandy loams and for wet land where few data were available. Miami and Carrington silt loams and loams and Kennan silt loam and loam classes included more resistant progenies than the average. Of all the strains, however, 85% were classified as mildew-free or resistant.

Frequency distributions for the several

TABLE 11

NUMERICAL FREQUENCIES OF STRAINS OF POA PRATENSIS IN DISEASE-REACTION CLASSES WHEN GROUPED ACCORDING TO SOIL AREA, LENGTH OF GROWING SEASON, AND GEOGRAPHIC AREA WHERE COLLECTED IN WISCONSIN

Classification	Mildew					Rust					Leaf spot			
	0	1	2	3	4	0	1	2	3	4	0	1	2	3
Soil areas*														
Knox (silt loams and loams)	45	31	9	2	3	5	39	39	21	2	21	28	12	2
Miami and Carrington (silt loams and loams)	36	10	1	3	....	2	24	20	7	1	20	15	3	1
Miami and Carrington (sands and sandy loams)	1	2	2	....	....	1	6	2	3	....	....	....	....	....
Superior (clays and clay loams; northern area)	11	6	3	1	1	....	16	16	5	2	6	14	7	1
Superior (clays and clay loams; southern area)	4	....	....	....	....	....	3	....	1	....	2	2	....	....
Sands	26	24	8	1	....	1	56	27	6	1	17	22	7	5
Spencer	17	15	....	....	1	....	8	15	13	1	7	14	4	5
Kennan (silt loams and loams)	20	4	1	1	1	4	18	9	4	....	5	24	2	2
Kennan (sands and sandy loams)	1	3	3	....	....	2	7	1	....	....	....	....	....	....
Peat	....	....	2	....	....	....	1	1	2	1	....	1	....	....
Wet land	....	4	2	1	....	....	3	4	....	....	....	1	....	....
Total	161	99	31	9	6	15	181	134	62	8	78	121	35	16
Length of growing season														
90–100 days	1	....	....	....	....	....	1	2	....	....	....	2	1	....
100–110 days	10	2	....	....	1	2	7	5	1	....	1	10	2	1
110–120 days	29	7	2	1	1	....	35	21	2	3	19	18	9	1
120–130 days	34	19	4	1	1	5	29	32	24	2	12	42	13	7
130–140 days	13	9	8	3	....	5	28	10	4	....	4	19	1	1
140–150 days	19	11	6	1	....	2	28	16	10	2	9	9	2	1
150–160 days	41	28	6	1	3	5	29	32	24	....	24	24	8	5
160–170 days	4	10	2	....	....	2	23	4	1	....	1	3	....	....
170–180 days	8	....	1	1	....	3	7	4	....	....	6	2	....	....
Total	159	86	29	8	6	24	187	126	66	7	76	129	36	16
Geographic areas														
Eastern Ridge and Lowlands	53	7	2	2	....	7	29	19	15	1	22	18	2	5
Central Plains	45	8	6	3	....	6	39	15	2	1	19	17	1	1
Western Upland	55	37	12	1	3	7	47	36	18	....	15	21	7	2
Northern Highland	47	20	....	....	2	1	17	29	18	3	14	28	8	....
Lake Superior Lowland	19	2	1	....	2	1	9	9	2	1	7	5	1	....
Total	219	74	21	6	7	22	141	108	55	6	77	89	19	8

* Cf. table 2.

soil regions with respect to reaction to rust were very similar and approached a normal distribution. Types from Spencer soils were somewhat more rust-susceptible as a group. Many strains, including 45% of the total, were classified as resistant, while only 4% were noted as rust-free.

Classification for leaf-spot reaction indicated slight differences among soils areas as to behavior of strains. Lots from Kennan silt loams and loams were somewhat more resistant as a group. Approximately 80% of the total number of strains observed were of leaf-spot-free or only slightly infected types. This is essentially the same proportion as that observed for mildew reaction.

Data for classification of disease reaction according to length of the growing season where strains were collected show that general similarity existed among groups in reaction to mildew (table 11). The only exception to this occurred in lots from the 160–170-day area, in which intermediate reaction types were more frequent than in other groups. Classification for rust reaction showed prominent differences among growing-season groups. The 120–130- and 150–160-day classes had distributions tending more toward susceptibility than other groups which were, among themselves, very similar. Most seasonal group distributions approached normality with respect to leaf-spot reaction; that for the 170–180-day season included relatively more leaf-spot-free strains than others.

The disease reactions of strains grouped by geographic regions are given in table 11. Mildew reactions of the several regional classes were quite comparable, those from the Western Upland including a relatively lower proportion of mildew-free strains than the others. Some variations are indicated in the distributions for reaction to rust. They were relatively similar, however. Central Plains lots were somewhat higher in proportion of resistant types than the others, whereas the Northern Highland group was inclined more toward susceptible reactions. Classification for leaf-spot reaction indicated that the Western Upland and Northern Highland areas included proportionately fewer disease-free strains than other sections.

In considering the classification for disease reaction when strains have been grouped according to soil, length of growing season, and geographic areas, it should be noted that numbers of collections are frequently too small to permit critical comparisons. This is particularly evident for leaf-spot reaction.

Frequency distributions of reaction classes for mildew were highly variable for the several regions of the United States and were not normal in type. They were somewhat less variable for the soil and growing-season classifications of Wisconsin data but were not normal, in that populations included excessive percentages of mildew-free and resistant types. Reactions to rust among the various lots from United States and Wisconsin sources were more similar than were reactions to mildew. Very little difference was apparent among the geographic areas of the country. Somewhat more irregularity existed in frequency distributions of data from Wisconsin, particularly with respect to soil groups. Frequency distributions among Wisconsin strains for leaf-spot reaction were approximately normal except for an excess of the resistant types. Considering the diseases observed, there was little indication that the region or the habitat where collected had served to modify the population relative to disease reaction.

Discussion

These studies were an attempt to investigate further the nature of variability in *P. pratensis* and its possible relations to geographical and climatological factors. Though a seemingly large number of progenies have been observed, it is very certain that they cannot be considered as completely representative of the regions sampled. The number of collections made in Wisconsin is perhaps a reasonably adequate sample of that area. Until more extensive data are available, those presented and the generalizations made may contribute to the increasing fund of knowledge about Kentucky bluegrass.

Approximately 75% of all progenies showed some morphological variability. Since numbers of plants grown in each progeny were small, it is probable that many of the types presumed to be constant were potentially variable. The average percentage of aberrant plants was 16.5 when based upon the total number. Considering only those progenies exhibiting segregation, this value was 20.5%. These figures are somewhat higher than those obtained by other workers, though they are reasonably similar to the results presented by BRITTINGHAM (4). It is the view of the writers that they are representative for the collections studied. Thus *P. pratensis* might be described as reproducing generally by apomixis; this was also BRITTINGHAM'S conclusion. Sexual reproduction may play a more important role in maintaining genetic diversity, however, than has been thought previously. Since uniformity is perhaps of little practical advantage for turf and pasture purposes, reasonable conformity to a parental type might suffice for maintenance and utilization of any original biotype selected.

There is some evidence in the results obtained that ecological conditions have served to allow the development of certain regional differences in the general relation of apomictic and sexual reproduction. This is not in agreement with the observations reported by BRITTINGHAM. Whether such differences were generally significant, however, could not be determined. Lowest percentages of aberrants appeared in lots from interior regions in the United States.

Comparisons were made of the relative morphological variability among regional collections. Although minor differences have been indicated, it would appear that regional and local influences have not served to isolate readily recognizable, morphological type-complexes. These results are in accord with those of ÅKERBERG (2), who reported that form-complex studies of *P. pratensis* did not indicate that Eu-*pratensis* was confined to any particular locality. He also observed that the Eu-*pratensis* forms were highly variable and widely scattered. Although the data are not strictly comparable, these results do not appear to support the conclusions of WELLHAUSEN and WEIBEL (15).

Leafiness, late vigor, and earliness of maturity appeared to show more regional differentiation than did other characters in the collections from various parts of the United States. Data for Wisconsin collections indicated that these same characters were highly variable, though leafiness was less so. Early vigor, growth habit, aggressiveness, and plant height did not show prominent regional characteristics. Disease reactions were not of value in differentiating regional classes, though some variation occurred.

Certain general impressions were obtained of progenies growing in the field. Those from the North Pacific and Northeastern regions of the United States ap-

peared to be more leafy, darker green in color, and more generally attractive from a plant-breeding viewpoint. They appeared also to be more generally variable in type within groups. Collections from the Central Plains were relatively less leafy, more prostrate, and more generally similar than those from other sections.

Among collections made in Wisconsin, those from the vicinity of Madison and from the Lake Superior Lowland were most variable and included more attractive types.

It might be concluded that collection of seed of *P. pratensis* from regions differing widely with respect to soil and climatic factors has not greatly increased the probability of obtaining increased variability as compared with more intensive collection in a single locality. Though some evidence contrary to this generalization has been presented, the data obtained are thought to be only suggestive and insufficient to establish this point definitely. When the Wisconsin collections were grouped, particularly on the basis of soil and length of growing season where obtained, greater type diversity was observed.

Summary

1. Eight hundred progenies derived from single panicles of *Poa pratensis* collected in twenty-seven states of the United States were studied in the seasons 1941–1944 at Madison, Wisconsin. These included 541 Wisconsin collections. All were grown in spaced nursery rows.

2. Progenies from the United States were classified according to the geographic region from which the parent seed was collected. Observations were obtained for percentage of aberrant plants, early vigor, growth habit, aggressiveness, leafiness, maturity, late vigor, plant height, and reactions to powdery mildew (*Erysiphe graminis*), leaf rust (*Puccinia poae-sudeticae*), and leaf spot (*Helminthosporium vagans*). Progenies from Wisconsin sources were grouped according to soil area, length of the growing season, and geographic area where parental seed was collected.

3. Among all progenies, 75.8% included some aberrant plants. The average percentage of segregation based upon the total progeny number was 16.5. When only segregating progenies were included, this value was 20.5%.

4. Differences among regions of the United States in percentage of segregation were generally large. No close association of such differences with regional, soil, or climatological variations could be established, although interior or continental regions showed generally lower percentages of aberrant types.

5. Collections grouped by regions of the United States were generally similar to one another in variation of early vigor, growth habit, and aggressiveness. Leafiness, late vigor, and relative maturity varied more extensively among regional groups. Lots from the East Central region were generally less aggressive; those from the Northeastern and North Pacific areas were more leafy; collections from the Northern Plains and the Northern Great Basin were of earlier maturity; and progenies from the Northern Plains and Central Plains were relatively more vigorous.

6. Differences among progenies derived from the various soil areas in Wisconsin were generally small. When grouped according to the length of the growing season in the locality of collection, significant differences in percentages of segregation were found, with the 140–150-day class having a high value. Strains from areas having longer growing seasons were generally somewhat higher in percentages of aberrants than those from areas of shorter seasons. The com-

parison by geographical regions indicated that the Eastern Ridge and Lowlands area and the Western Upland area of Wisconsin were significantly higher in percentages of aberrants than were other areas.

7. Classifications of Wisconsin collections for morphological characters showed a generally great diversity for behavior among lots from different soil areas. Frequency distributions were similar for early vigor and leafiness for the different soils and were somewhat less so for growth habit; those for aggressiveness, maturity, and late vigor were highly dissimilar. Based upon average length of the growing season, distributions for the several characters were generally comparable with those for the soil classification. Frequency distributions for early vigor, growth habit, and leafiness were most similar. The classification for maturity showed least relation to length of growing season. Grouping on the basis of geographical areas resulted in frequencies relatively similar to those for soil and growing seasons. Classification by plant height suggested no particular relationships to region of origin in the United States. In the Wisconsin collections analysis by soil and geographic regions did not indicate prominent differences in relative frequencies of plants with respect to height. When grouped by growing seasons, the 100–110- and 130–140-day lots exhibited relatively lower frequencies of intermediate types, though this was of questionable significance.

8. Collections from the United States varied irregularly in frequency distributions for reaction to powdery mildew. Lots from different regions appeared to differ markedly in proportionate frequencies of resistant and susceptible types. Approximately 34% of the progenies were observed to be mildew-free, whereas 12% were very susceptible. Reaction to leaf rust was generally similar among groups of strains from the various regions of the United States. Of the progenies, 14% were classified as rust-free and 66% as resistant.

9. Classification of Wisconsin collections with respect to soil of origin showed certain prominent differences to exist among soil areas in the character of the distributions among disease-reaction classes. In general, however, distribution for the three kinds of diseases were similar. Considering reaction to leaf rust, collections from certain heavier soil areas were relatively more susceptible to disease than those from others. When classified according to length of the growing season, little relation was shown between length of season and relative frequencies of reaction types, though occasional exceptions occurred. No consistent relation between length of growing season and relative disease resistance was found. Collections grouped according to geographic regions exhibited no significant differences in reaction to diseases.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ÅKERBERG, E. Apomictic and sexual seed formation in *Poa pratensis*. Hereditas **25**:359–370. 1939.
2. ———. Cytogenetic studies in *Poa pratensis* and its hybrid with *Poa alpina*. Hereditas **28**:1–125. 1942.
3. ALLISON, J. L., and CHAMBERLAIN, D. W. Grass diseases in Wisconsin in 1941. Plant Dis. Rptr. **26**:19–22. 1942.
4. BRITTINGHAM, W. H. Type of seed formation as indicated by the nature and extent of variation in Kentucky bluegrass and its practical implications. Jour. Agr. Res. **67**:225–264. 1943.
5. BROWN, W. L. Chromosome complements of five species of *Poa* with an analysis of variation in *Poa pratensis*. Amer. Jour. Bot. **26**:717–723. 1939.

6. BROWN, W. L. The cytogenetics of *Poa pratensis*. Ann. Mo. Bot. Garden 28:493–522. 1941.
7. Climate and man. 1941 Yearbook, U.S. Dept. of Agriculture.
8. EDGECOMBE, S. W. Report of fine turf research at Iowa State College. Trans. Iowa State Hort. Soc. Pp. 320–329. 1940.
9. FENNEMAN, N. M. Physiographic divisions of the United States. 3d ed. Annals Assoc. Amer. Geographers 18:261–353. 1928.
10. MARTIN, L. The physical geography of Wisconsin. 2d ed. Wis. Geol. and Nat. Hist. Surv. Bull. 36. 1932.
11. MUSGRAVE, R. B. Life history studies of *Poa pratensis* L. Unpublished Doctor's diss. Univ. Illinois. 1940.
12. MYERS, W. M. Second-generation progeny tests of the method of reproduction in Kentucky bluegrass, *Poa pratensis* L. Jour. Amer. Soc. Agron. 35:413–419. 1943.
13. SMITH, D. C., and NIELSEN, E. L. Morphological variation in *Poa pratensis* L. as related to subsequent breeding behavior. Jour. Amer. Soc. Agron. 37:1033–1040. 1945.
14. TINNEY, F. W., and AAMODT, O. S. The progeny test as a measure of the types of seed development in *Poa pratensis* L. Jour. Hered. 31:457–464. 1940.
15. WELLHAUSEN, E. J., and WEIBEL, R. O. Genotypes of bluegrass in good and poor permanent pastures. Abstract paper presented at annual meeting, Amer. Soc. Agron., St. Louis, Mo. 1942.
16. WHITSON, A. R. Soils of Wisconsin. Wis. Geol. and Nat. Hist. Surv. Bull. 68. 1927.

REACTIONS OF DECAPITATED BEAN PLANTS TO CERTAIN OF THE SUBSTITUTED PHENOXY COMPOUNDS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 577

J. M. BEAL

Introduction

The histological reactions of bean plants to certain of the substituted phenoxy compounds applied to the bases of the heart-shaped leaves have recently been described (1). In general, the second internodes of these plants showed tissue responses similar to those induced in the bean by a number of other compounds applied to the cut curface of the young second internode following its decapitation just below the first trifoliate leaf (4, 6). Although all tissues of the second internode, except the epidermis, local areas of the outer cortex, and perhaps the pericycle, proliferated actively as a result of the application of the phenoxy compounds, no roots were developed in this internode during periods as long as 20 days following their application. The responses in the first internode and hypocotyl were somewhat more restricted as to tissues affected than was the case in the second internode. Usually in the first internode and hypocotyl only the endodermis, cambium, phloem, and ray parenchyma responded, but from the cambium, phloem, and ray parenchyma, especially from the two latter, adventitious roots developed in abundance.

Because many of the earlier histological studies had been made following application of the compounds to the cut surface of the second internode of decapitated bean stems, it was decided to apply the phenoxy compounds previously employed (1) to the cut surface of decapitated young second internodes of the same strain of Red Kidney beans as had been used in former experiments. The compounds were applied as 0.5% and

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

as 1% mixtures in both lanolin and Carbowax 1500 as carriers.

Red Kidney bean plants were grown in the greenhouse in flats containing rich loam soil under conditions simulating as closely as possible those used in the former study (1). The second internode was cut squarely across just below the node of the first trifoliate leaf as the leaf started to expand. Immediately afterward the cut surface was covered over with the lanolin or Carbowax mixture, and the plants, together with untreated controls, were kept under conditions favorable for vigorous growth.

Material for histological study was collected at regular intervals after treatment, fixed in Navashin's solution, handled according to the tertiary butyl-alcohol method, and sectioned at 10 μ.

Observations

Gross responses

The gross responses of the decapitated plants to 2-chlorophenoxyacetic, 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic (2,4-D), and 2,4,5-trichlorophenoxyacetic acids were, in many respects, different from those resulting from application of these compounds to the bases of the heart-shaped leaves. The visible responses in the decapitated plants were evident chiefly in the upper 10–12 mm. of the second internode near the point of application of the mixtures and in the leaves developed from the axillary buds. All the compounds retarded development of the axillary buds, with the least effects resulting from the application of 2-chlorophenoxyacetic acid, and all the compounds, except 2,4,5-trichlorophenoxyacetic acid, resulted in the formation of at least some dwarfed and often deformed and crinkled leaves on the axillary shoots. No epinasty and little or no stem curvature resulted from the applications.

Under high temperature and humidity the Carbowax mixtures often melted and ran down over the surface of the first internode and sometimes even as far as the upper part of the hypocotyl. Responses in the form of stem enlargement and often of root formation commonly followed. Nearly all the plants, however, to which the Carbowax mixtures were applied showed more or less injury at and near the place of application and frequently rather extensive death of the stem tissues (fig. 1*C*, *E*, *G*). The lanolin mixtures resulted in much less tissue injury (fig. 1*B*, *D*, *F*, *H*).

When Carbowax alone was applied to the apices of decapitated second internodes, slight shrinkage and evidences of partial dehydration of the cells occurred within 24 hours. At 72 hours following application many tiers of dead and shrunken cells were present at the apical ends of the treated internodes (fig. 2). Immediately below these cells was a zone consisting of several tiers of cells in which division had begun. A few layers of these cells adjacent to the dead tissue became suberized during the next few days and functioned as a protective layer over the distal end of the hypocotyl. The volume and area of dead cells resulting from the application of the Carbowax did not vary greatly and did not increase after the first 72 hours in a number of stems which were kept under observation for more than 2 weeks. Injury from the Carbowax alone was in no case as extensive as that from the substituted phenoxy compounds applied in Carbowax.

All the treated plants developed tumor-like swellings at or just below the point of application of the mixtures. The least responses were induced by 2-chlorophenoxyacetic acid (fig. 1*A*, *B*); 4-chloro-

phenoxyacetic (fig. 1*C*, *D*), 2,4-dichlorophenoxyacetic (fig. 1*E*, *F*), and 2,4,5-trichlorophenoxyacetic acids (fig. 1*G*, *H*) showed nearly the same total growth responses. None of the treated plants developed enlargements on the first internodes or hypocotyls except those plants on which the Carbowax mixtures melted and ran down the stems. The time and the rate of elongation of the axillary buds were delayed by all the mixtures, but least by the 2-chlorophenoxyacetic acid. Although the leaves produced by axillary buds on at least a part of all the treated plants showed dwarfing and deformity, except on those to which 2,4,5-

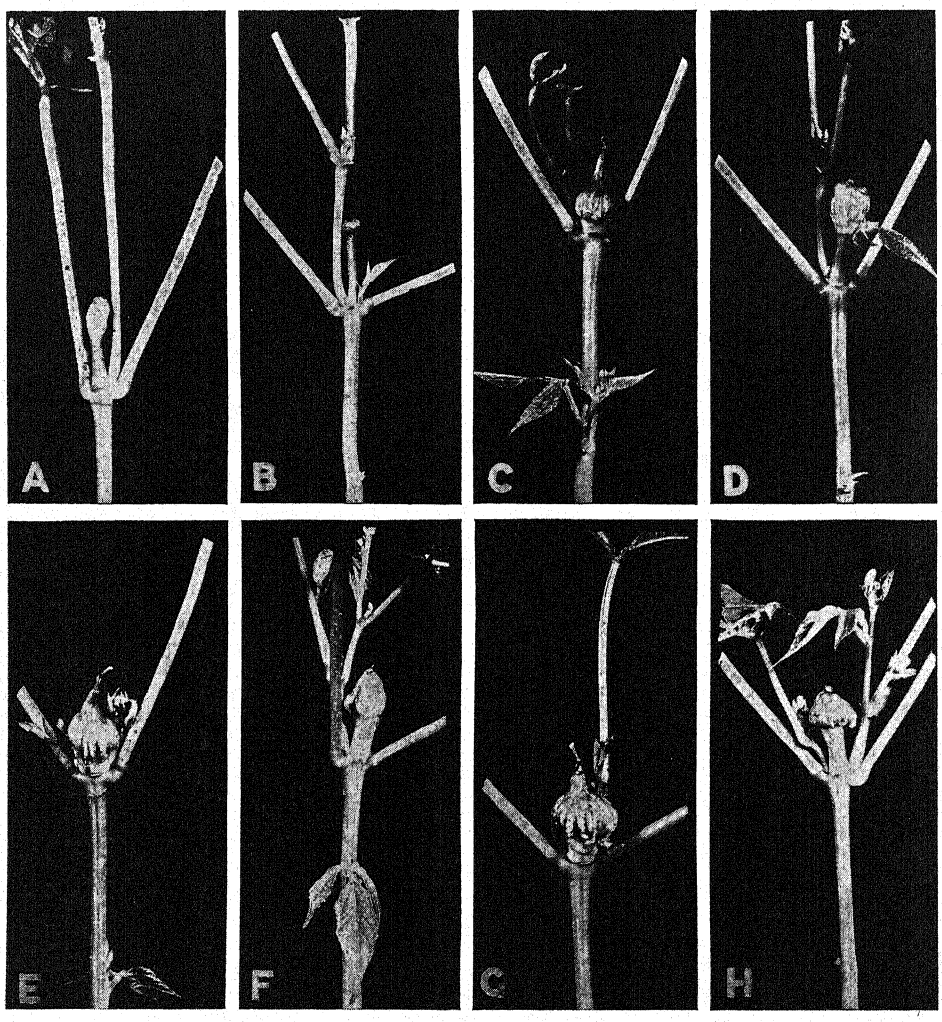


FIG. 1.—Effects of substituted phenoxy compounds on second internode of bean at 16 days following application. *A*, 2-chlorophenoxyacetic acid in Carbowax 1500; *B*, same in lanolin; *C*, 4-chlorophenoxyacetic acid in Carbowax; *D*, same in lanolin; *E*, 2,4-dichlorophenoxyacetic acid in Carbowax; *F*, same in lanolin; *G*, 2,4,5-trichlorophenoxyacetic acid in Carbowax; *H*, same in lanolin. Protruded roots evident in *E* and *G* but in no others.

trichlorophenoxyacetic acid had been applied, the effects were not nearly so pronounced as when the substances had been applied to the bases of the heart-shaped leaves.

Although slight color changes were apparent in the decapitated stems within 24 hours following application of the mixtures, little gross response was noticeable until 30 hours or more had elapsed. A faint yellowing of the tissues first became apparent within 24 hours just below the cut surface to which the mixtures had been applied. By the end of 48 hours the yellowing was more pronounced and the swelling greater. Enlargement continued steadily for several days, the tumors ordinarily reaching their ultimate size in 12–14 days. They then remained relatively constant in size for at least 2 weeks longer. Many, perhaps most, tumors attained a diameter of three to four times that of the basal half of the treated internode, except following application of 2-chlorophenoxyacetic acid; the latter tumors seldom reached a diameter twice that of the lower portion of the second internode (fig. 1*A*, *B*). Although root primordia were commonly developed, only a few of the treated stems ever showed protruded root tips (fig. 1*E*, *G*), and then only following treatment with 2,4-D and 2,4,5-trichlorophenoxyacetic acid in Carbowax.

Histological changes

Definite responses were apparent in sections of the second internode to each growth-regulating substance 24 hours after application, with the least responses following the application of 2-chlorophenoxyacetic acid. This substance applied in lanolin usually induced little response—often none. When Carbowax was the carrier, responses were commonly evident in 24 hours, although some stems responded so little that practically no swelling could be detected, and sections showed little effect even after 72 and 96 hours. Because of the slight responses, no photomicrographs of these stems are included.

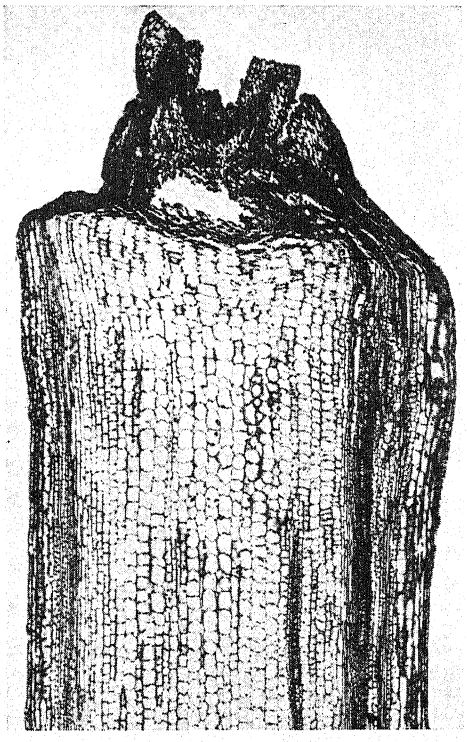


Fig. 2.—Longisection of decapitated second internode 72 hours after apical treatment with Carbowax alone. Many tiers of cells killed and shrunken at and below point of application, with several layers adjacent to these beginning to divide. Tissues at right side show effects of Carbowax which melted and ran over surface of stem.

Responses to applications of 4-chlorophenoxyacetic acid in both Carbowax and lanolin were generally evident at 24 hours, although the responses were much greater to the lanolin mixtures (fig. 3*B*). The cortical cells had elongated radially; their cytoplasmic content had become denser. Many endodermal cells had divided tangentially. Active divisions had

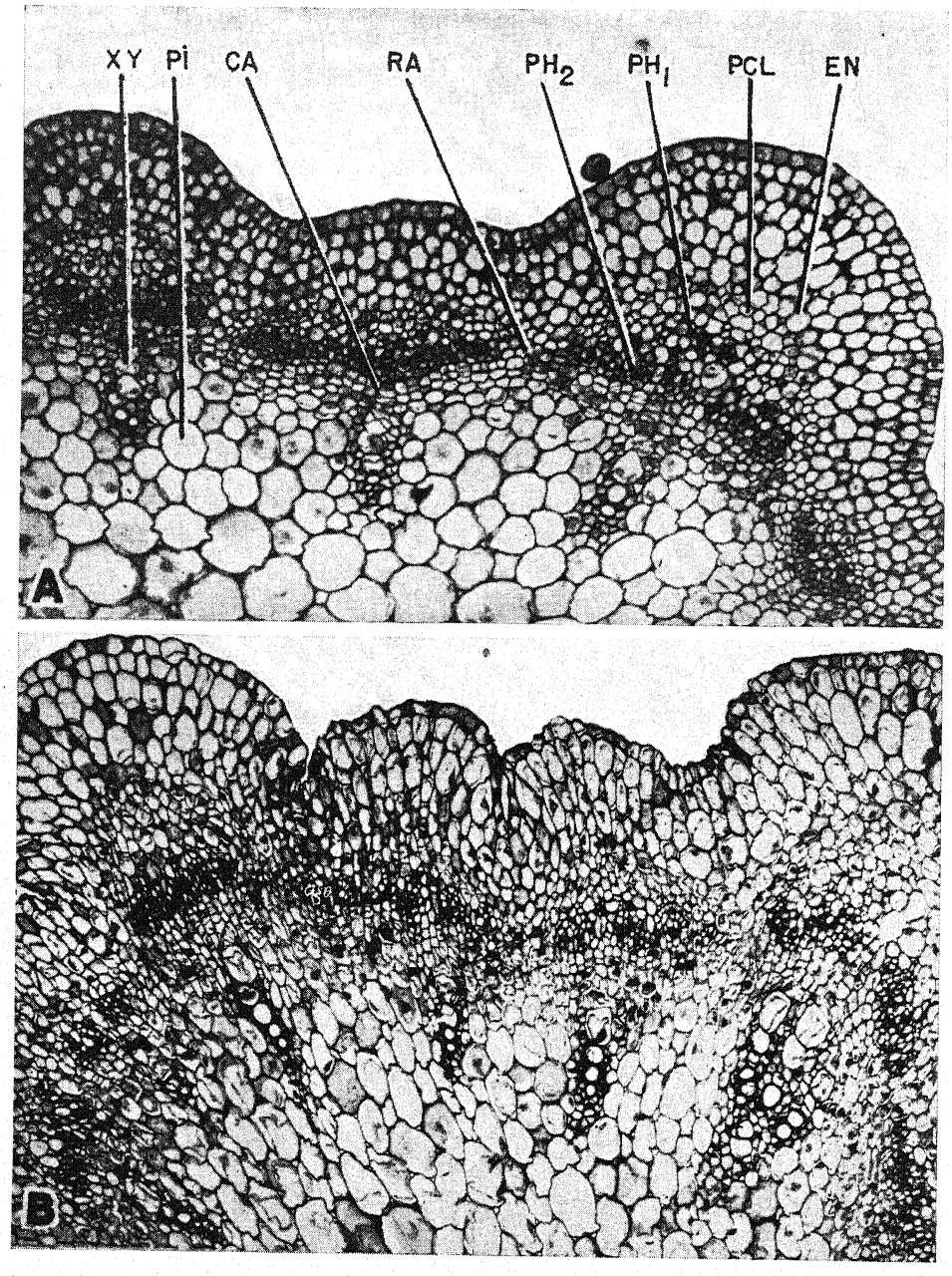


FIG. 3.—*A*, transection near top of second internode of bean at time of treatments, showing various tissues and stage of development (*xy*, xylem; *pi*, pith; *ca*, cambium; *ra*, ray; ph_2, secondary phloem; ph_1, primary phloem; *pcl*, pericycle; *en*, endodermis). *B*, second internode 24 hours after treatment with 4-chlorophenoxy-acetic acid in lanolin; activity beginning in endodermis, cambium, and ray parenchyma, with only slight activity in phloem parenchyma; cortical and pith cells radially elongated.

started in the cambium, phloem, and ray parenchyma cells, but the pericycle appeared unaffected. Although the cells of the pith showed radial elongation, there were no evidences of increased meristematic activity.

Only slight response resulted at 24 hours from applications of 2,4-D in Carbowax. Although the cortical cells showed radial elongation, little effect was evident in endodermis, phloem, cambium, ray parenchyma, or pith (fig. 4*A*). In contrast, application of this compound in lanolin resulted in division of some cells of the endodermis, with considerable activity in the cambium and ray parenchyma and a smaller amount in the phloem. The pith cells showed radial elongation but apparently no increase in the rate of divisions (fig. 4*B*).

At 24 hours essentially no changes could be detected from application of 2,4,5-trichlorophenoxyacetic acid in Carbowax (fig. 5). Applied in lanolin, however, this substance induced marked responses in the cambium and ray parenchyma, with less in the phloem. Cells of the cortex, endodermis, pericycle, and pith, except for radial elongation, were relatively unchanged (fig. 5*B*). The epidermis was unresponsive to all the compounds in these experiments.

At 33 hours the responses were much more evident. Proliferation of endodermis, cambium, phloem, and ray parenchyma had continued, although at a greater rate in some stems (figs. 6*A*, 7*B*) than in others (figs. 6*B*, 7*A*). In general, however, the early responses occurred at a more rapid rate in those stems to which the growth-regulating substances had been applied in lanolin as the carrier.

At 72 hours the responses were similar to all the compounds (figs. 8*A*, *B*, 9*A*, *B*), except 2-chlorophenoxyacetic acid, which commonly induced relatively little response. The total amount of activity had been greater in the internodes to which 2,4-D and 2,4,5-trichlorophenoxyacetic acid were applied, especially when lanolin had been used as the carrier. Definite root primordia were not yet evident.

At 144 hours the stems to which 2,4-D and 2,4,5-trichlorophenoxyacetic acid had been applied in Carbowax showed well-developed adventitious root primordia (figs. 10*A*, 11*A*), but the stems to which the same substance had been applied in lanolin had not developed root primordia (figs. 10*B*, 11*B*), although some suggestions of root primordia showed (fig. 11*B*); 4-chlorophenoxyacetic acid had not stimulated root formation when applied in either carrier. The induced roots originated in the pith rays, where they may occur in one to several of the rays, as reported for their origin in the first internodes in the previous study (1).

Some second internodes to which 4-chlorophenoxyacetic and 2,4-dichlorophenoxyacetic acids were applied in Carbowax showed well-formed roots internal to the cambium at 17 days following application of the mixtures (figs. 12*A*, 13*A*). This development has not been reported heretofore for the bean following treatment with any growth-regulating substance, although it has been described for the tomato (2), *Mirabilis* (3), and *Iresine* (5). Roots external to the cambium were not induced by 4-chlorophenoxyacetic acid applied in Carbowax (fig. 12*A*), nor were well-formed normal-appearing root primordia developed when this substance was applied in lanolin (fig. 12*B*). In spite of the earlier marked proliferation following after the application of all these compounds in lanolin as a carrier, the Carbowax mixtures had induced greater total responses by the sixth day. This advantage was not lost

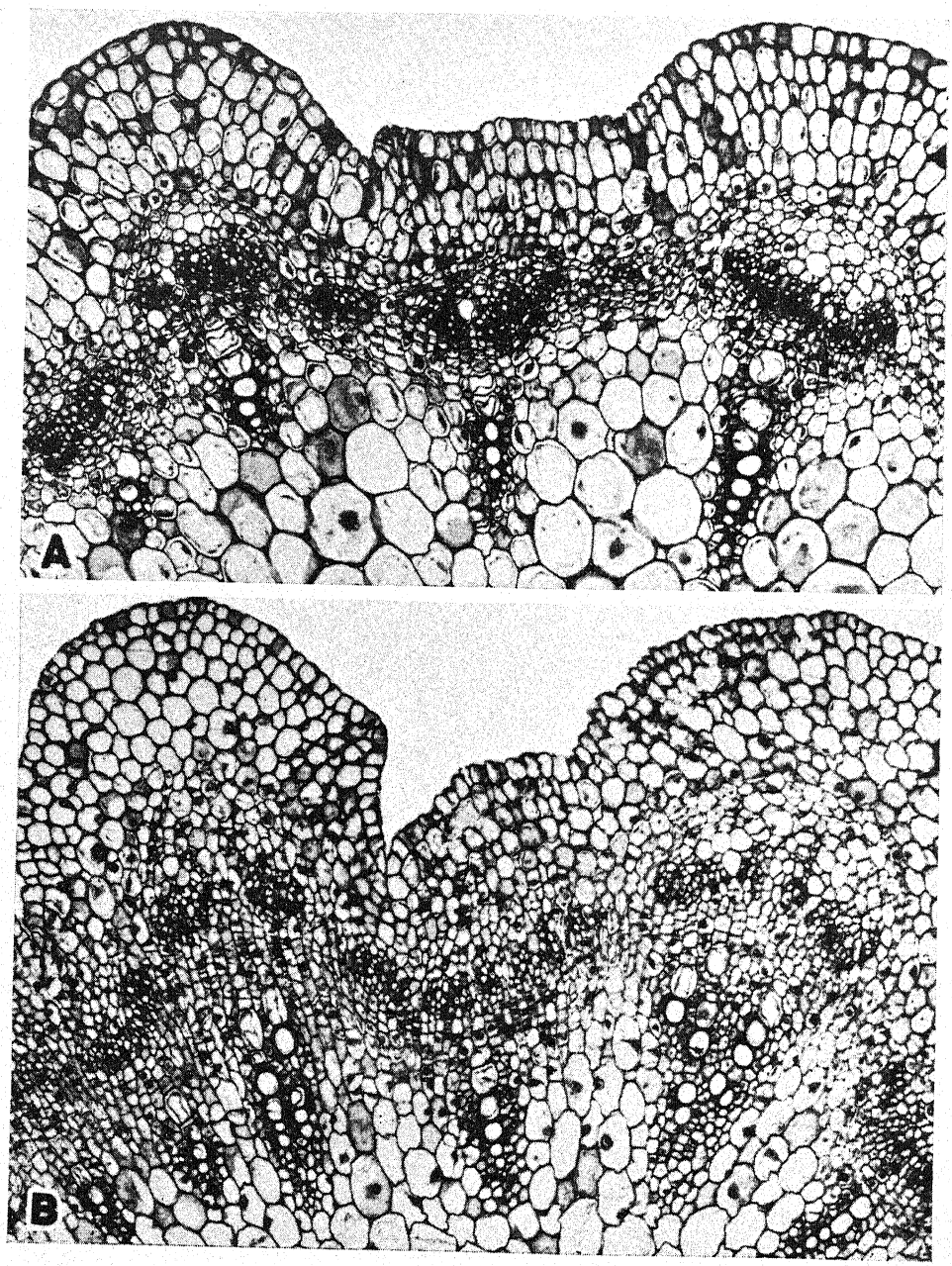


FIG. 4.—Second internode 24 hours after apical treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax, showing practically no induced activity; and *B*, in lanolin, showing slight activity in endodermis and phloem parenchyma and fairly pronounced activity in cambium and ray parenchyma. *A* magnified slightly more than *B*.

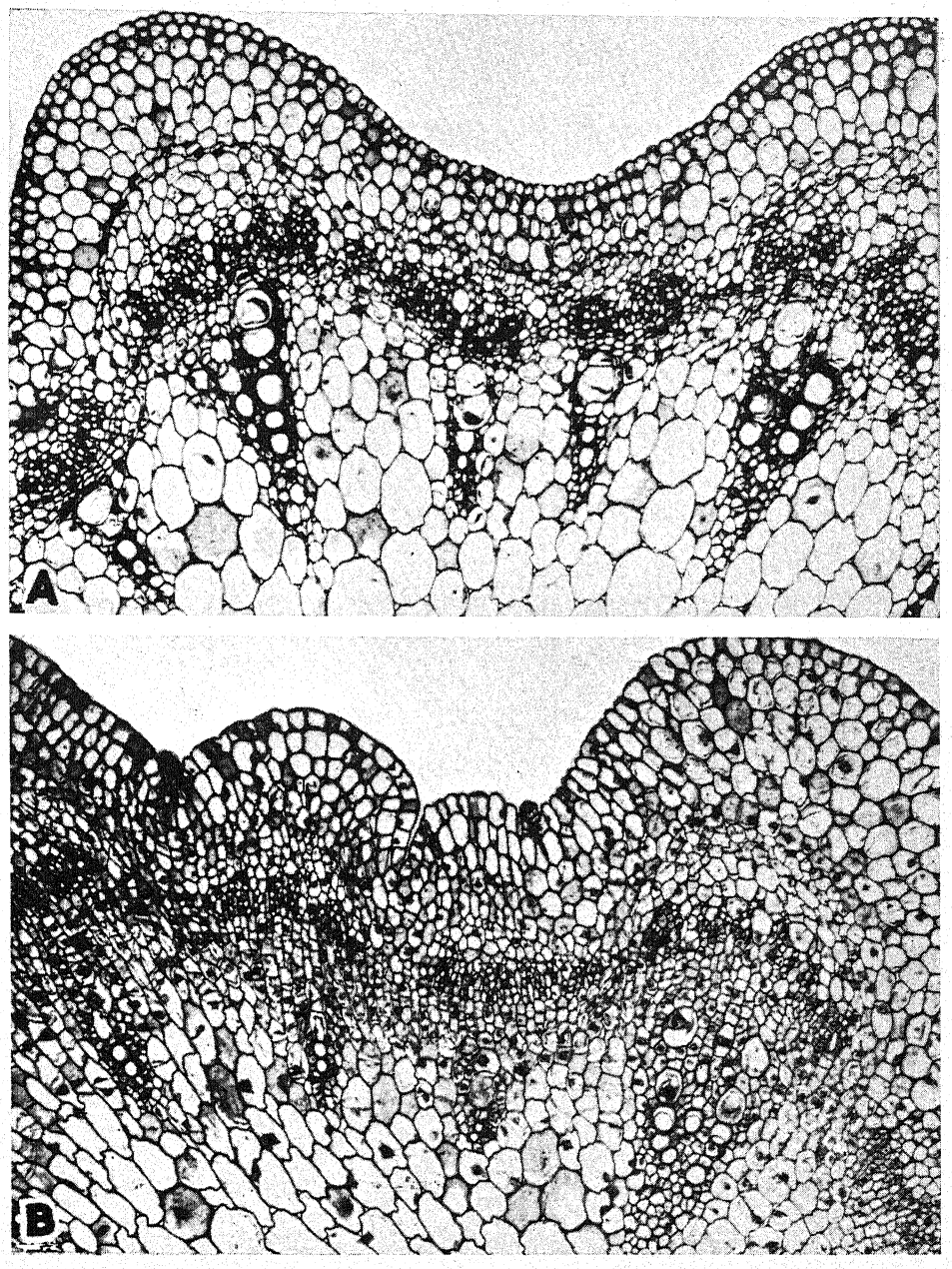


FIG. 5.—Second internode 24 hours after apical treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax, showing little or no induced activity. *B*, in lanolin, showing activity beginning in endodermis and phloem parenchyma and greater responses in cambium and ray parenchyma. Pith and cortex apparently unaffected.

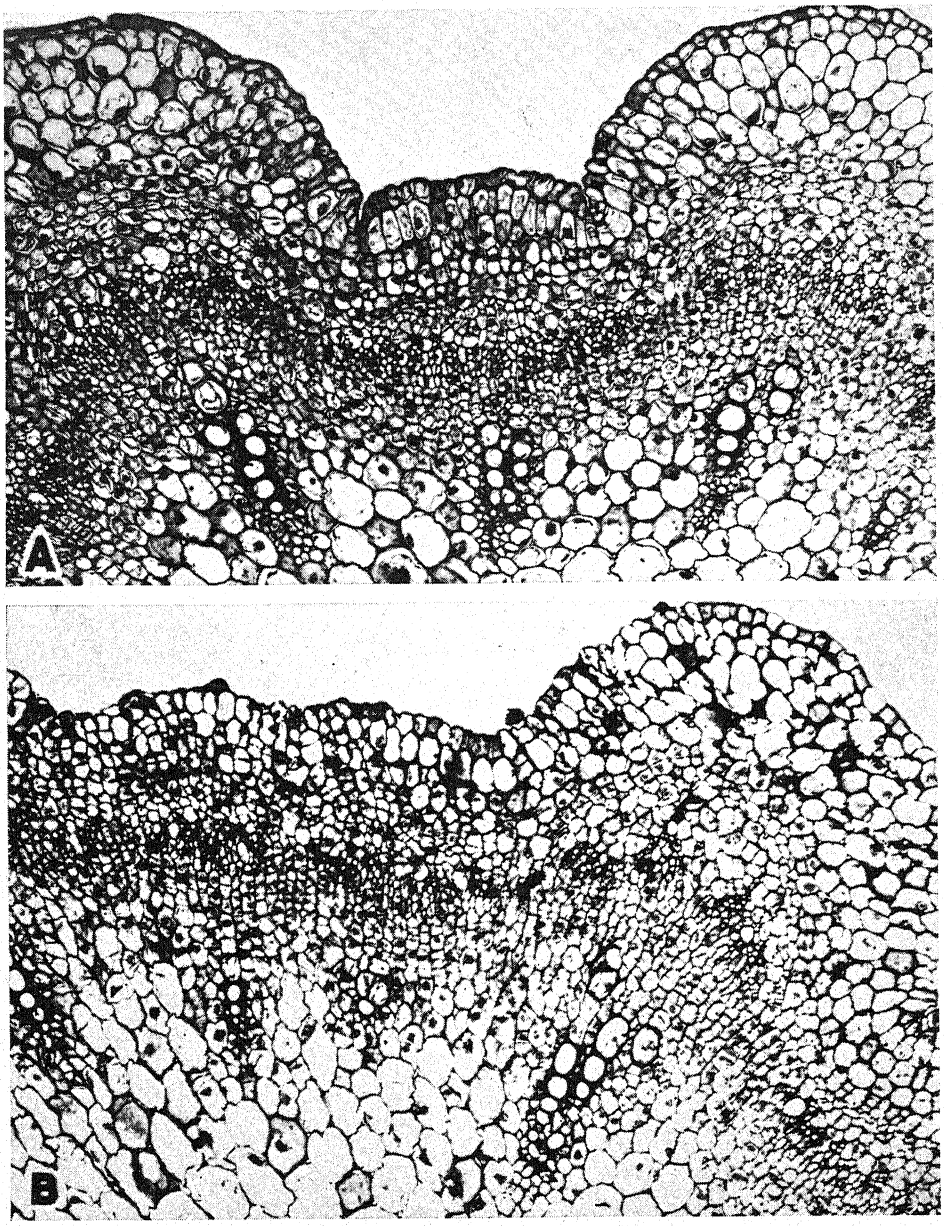


FIG. 6.—Second internode 33 hours after apical treatment: *A*, with 4-chlorophenoxyacetic acid in lanolin, showing responses in endodermis, cambium, and ray parenchyma; little response in phloem. *B*, 2,4,5-trichlorophenoxyacetic acid in lanolin. Same tissues have responded as in *A*.

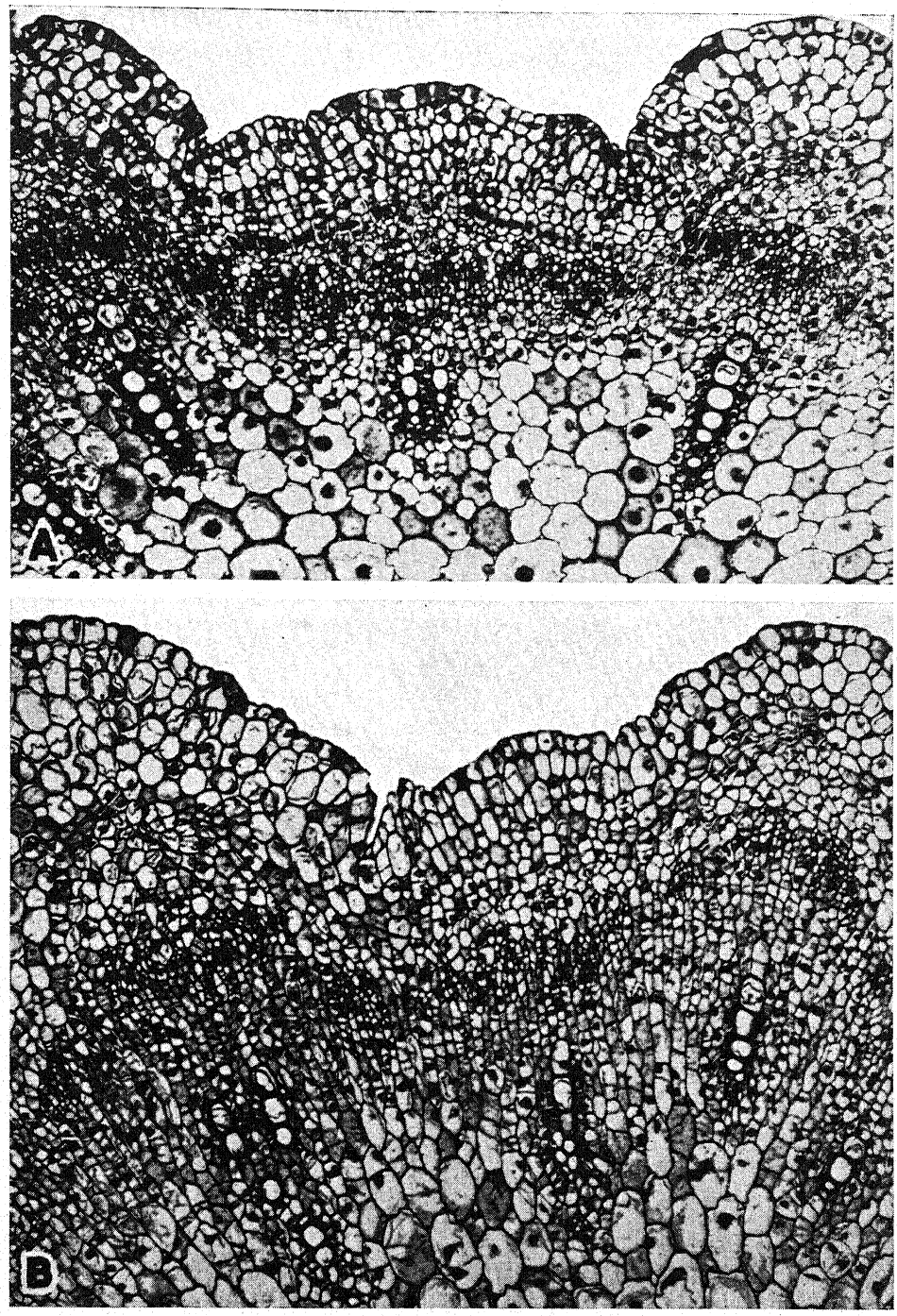


FIG. 7.—Second internode 33 hours after apical treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax, showing only slight responses in endodermis, cambium, phloem, and ray parenchyma. *B*, in lanolin, showing greater activity in all these tissues, especially in cambium and ray parenchyma.

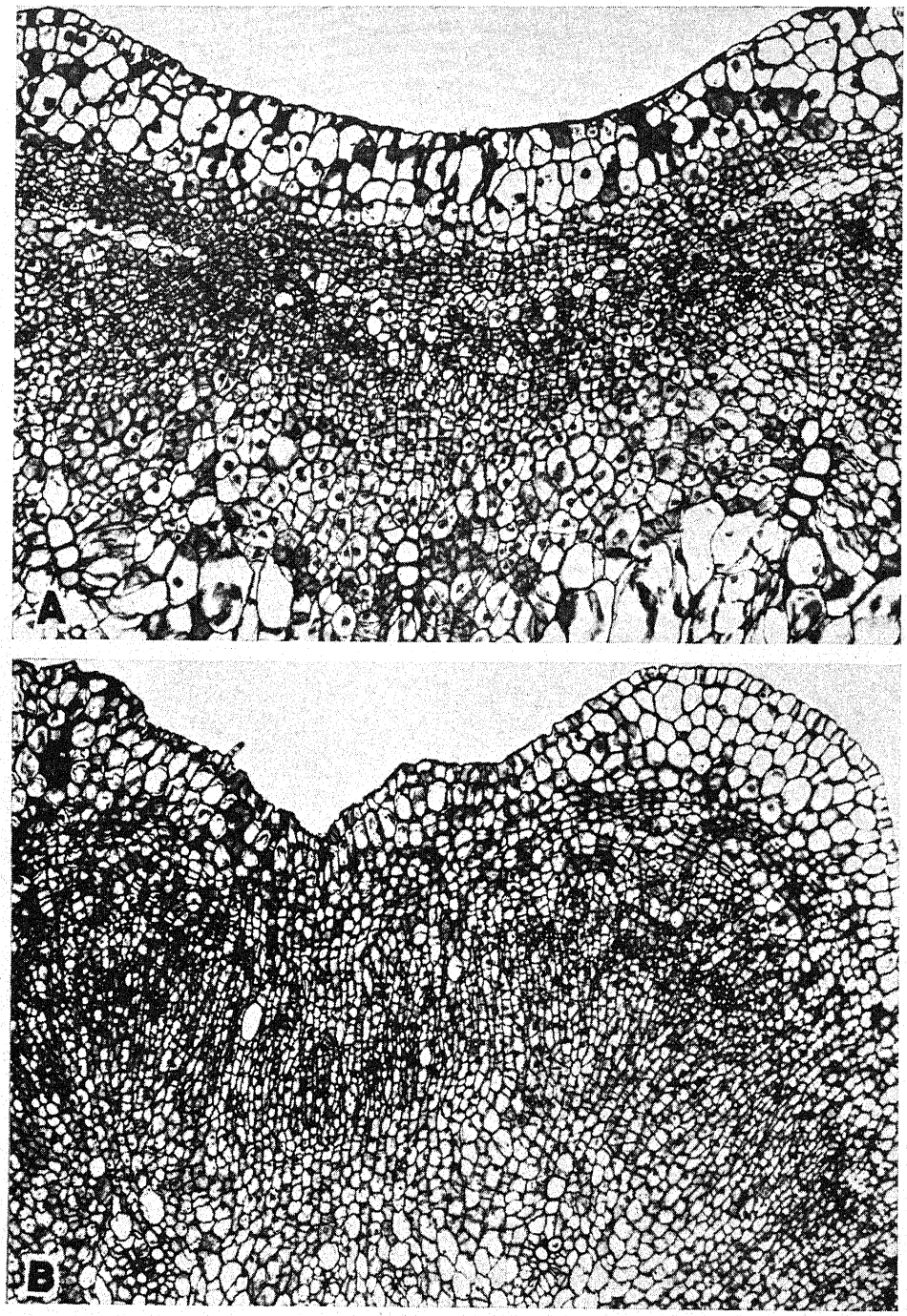


FIG. 8.—Second internode 72 hours after apical treatment: *A*, with 4-chlorophenoxyacetic acid, showing activity in endodermis, cambium, phloem, and ray parenchyma; outer cortical and some pith cells enlarged. *B*, with 2,4,5-trichlorophenoxyacetic acid in lanolin, showing marked proliferation of endodermis, cambium, phloem, and ray parenchyma. Outer pith cells and a few inner cortical cells have been responsive.

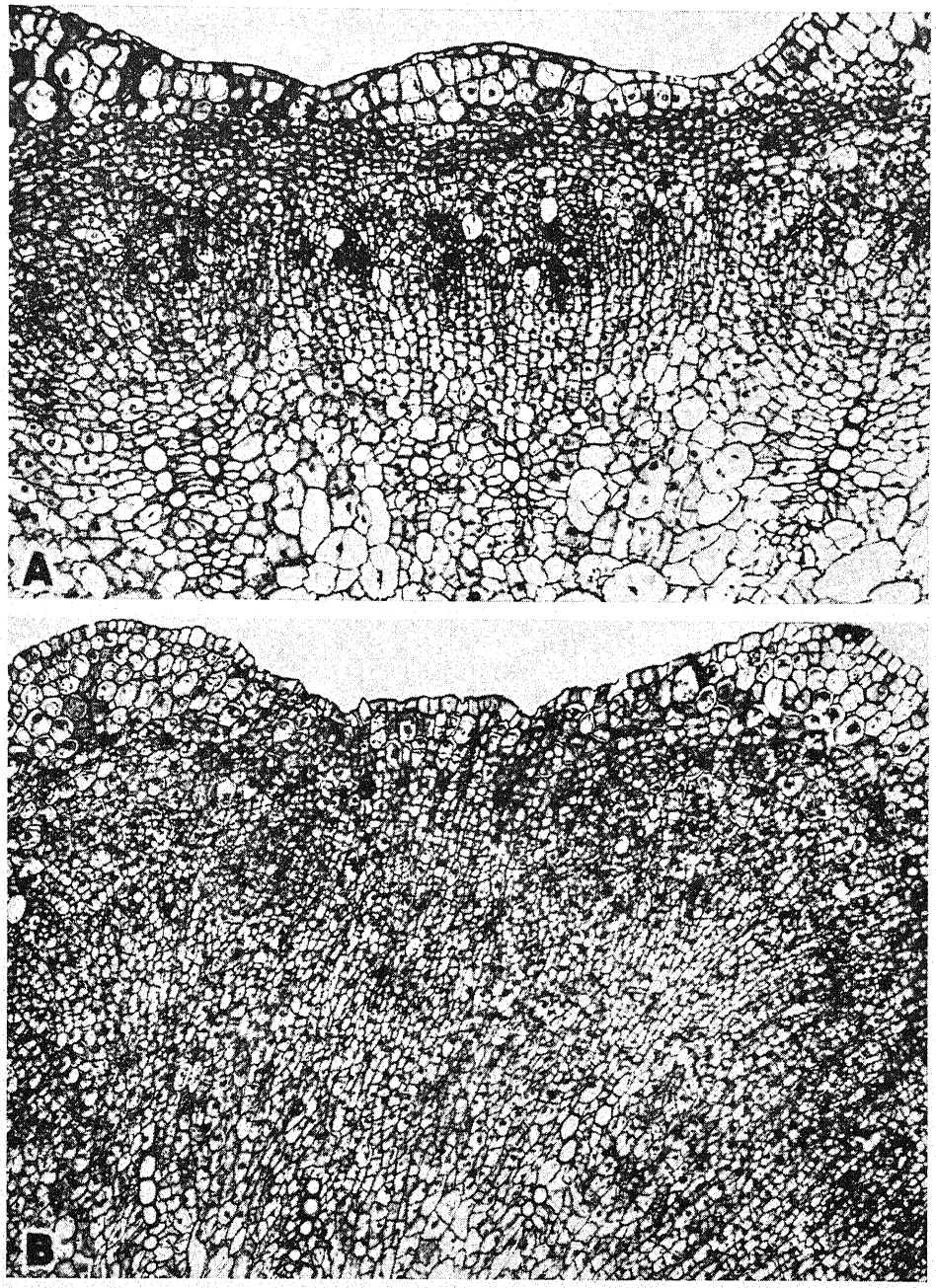


FIG. 9.—Second internode 72 hours after apical treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax. *B*, in lanolin. Same tissues respond in both stems, but rates and degree of response are different. Cambium, ray, and phloem parenchyma much more active in *B*, although endodermis has responded essentially the same in both. Inner cortex slightly active in both.

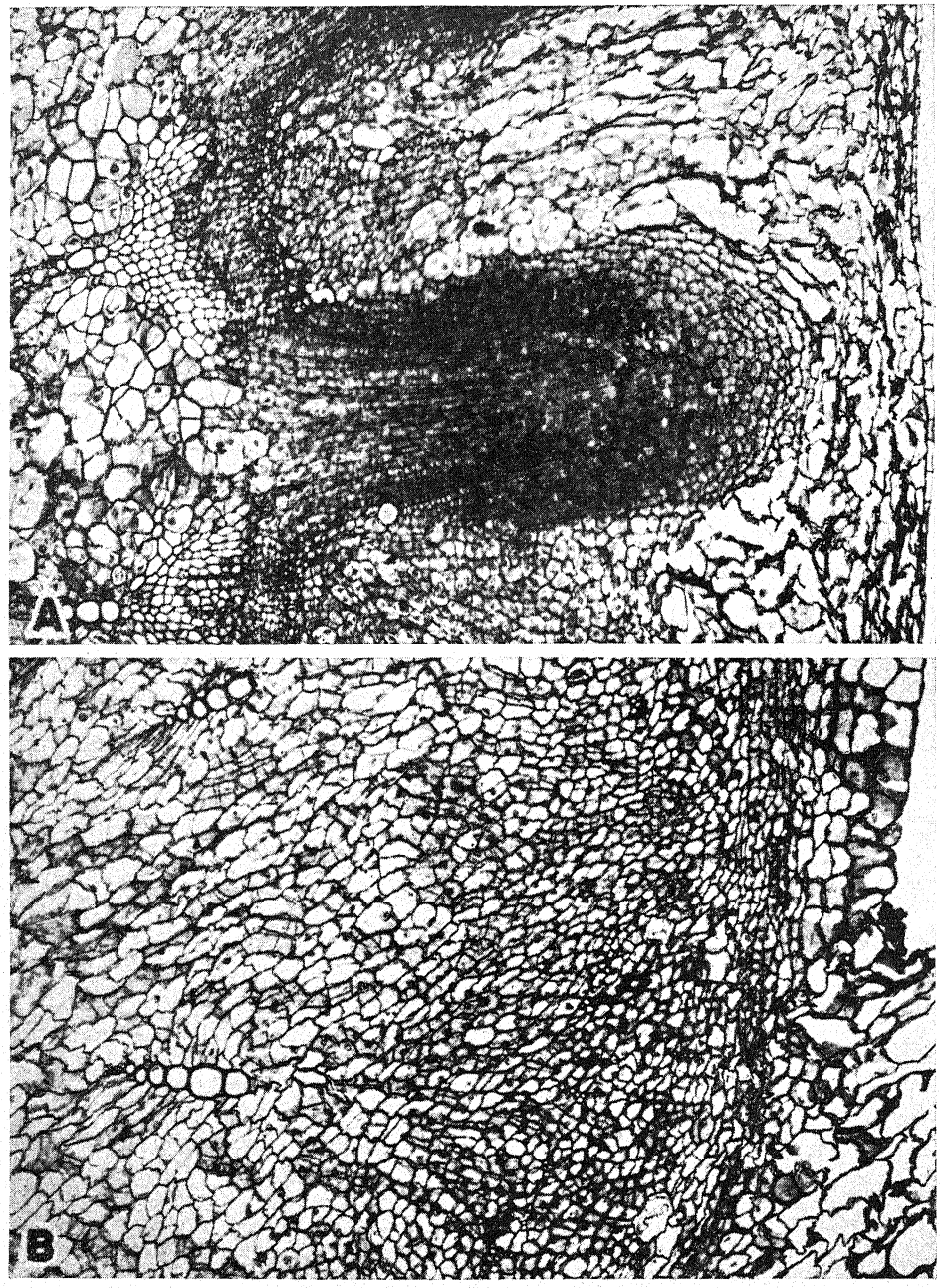


FIG. 10.—Second internode 144 hours after apical treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax. *B*, in lanolin. Stems treated with Carbowax mixture now show much greater responses, with well-developed root primordia in pith rays, while those treated with lanolin mixture have responded relatively little more than at 72 hours. (Cf. figs. 9*B* and 10*B*.)

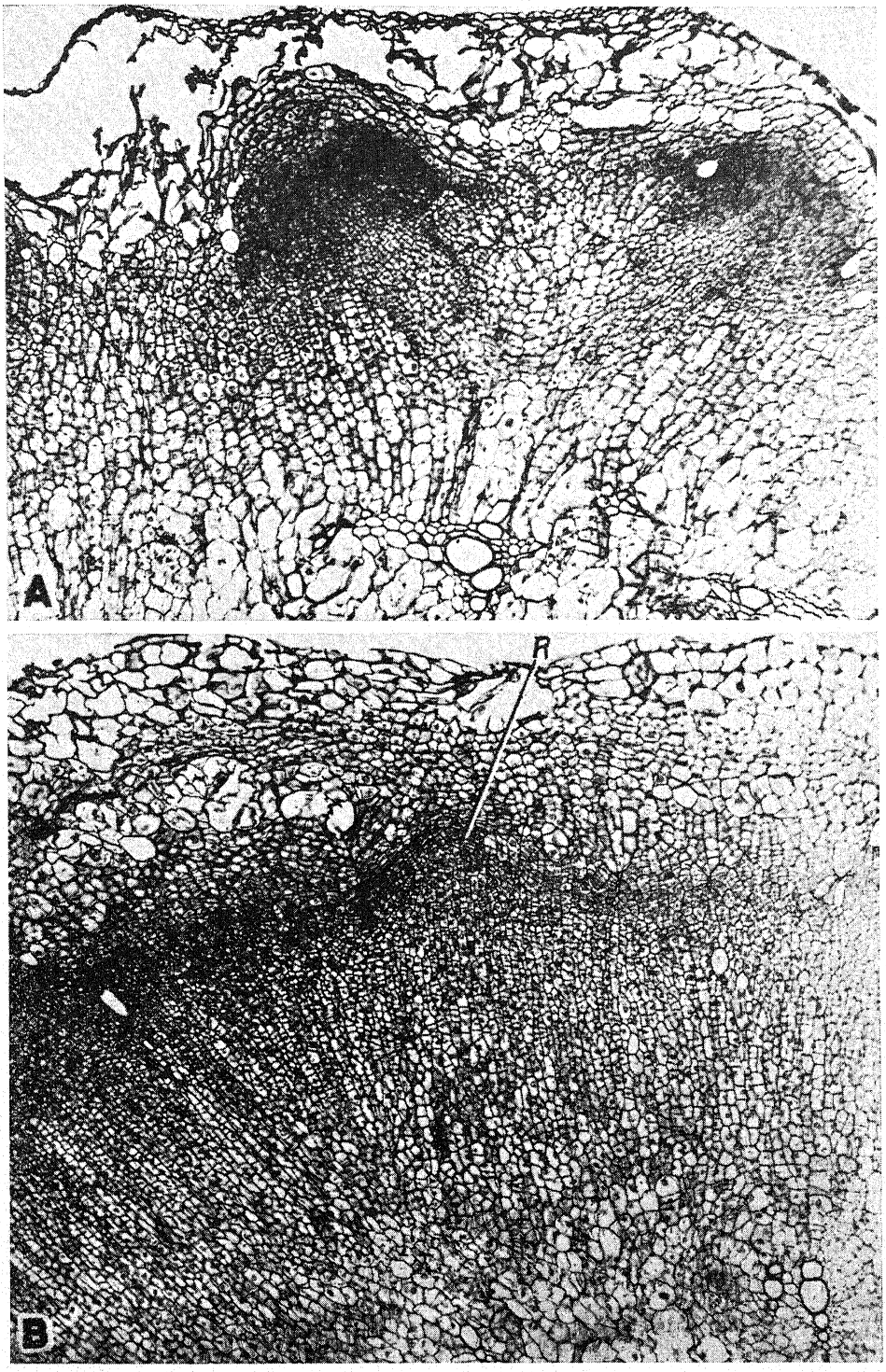


FIG. 11.—Second internode 144 hours after apical treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax. *B*, in lanolin. Tissues involved and their responses similar to those in fig. 10*A* and *B*, respectively. What appears to be a developing root primordium at top center of *B* does not develop into a well-organized root (see fig. 14*B*). Endodermis and cortex chiefly crushed and dead in figs. 10*A* and 11*A*.

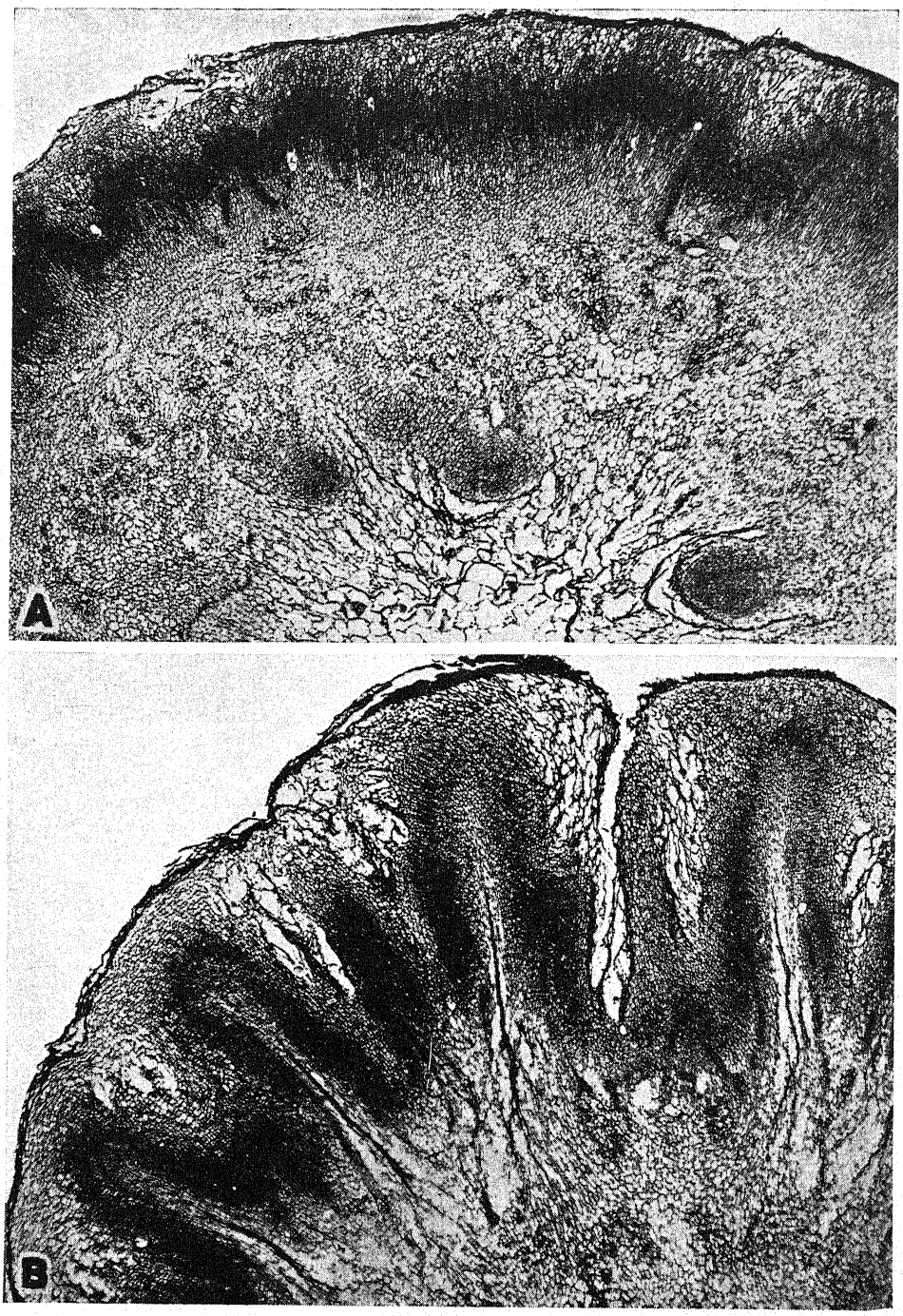


FIG. 12.—Second internode 17 days after apical treatment with 4-chlorophenoxyacetic acid: *A*, in Carbowax, marked activity throughout most of stem; a few adventitious roots developed in pith, but no well-organized roots developed external to cambium. *B*, in lanolin, showing neither internal roots nor well-organized external ones; although considerable proliferation occurs in endodermis, phloem, cambium, and ray parenchyma, well-developed root primordia were never organized. Death of cells in some tissues has begun.

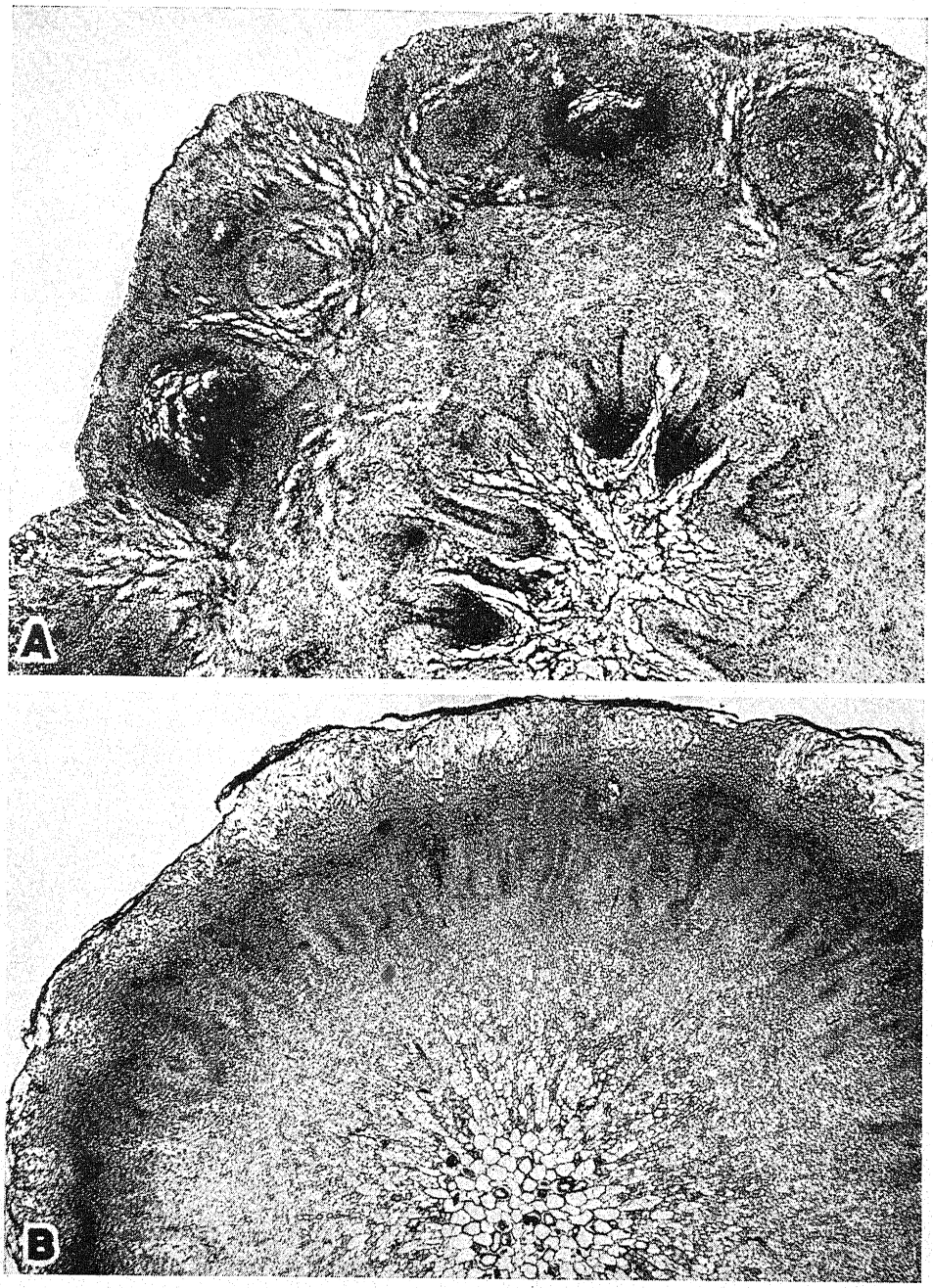


FIG. 13.—Second internode 17 days after apical treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax, showing well-organized internal adventitious roots and less well-organized ones external to cambium. *B*, in lanolin, showing marked proliferation throughout, except in central pith, pericycle, outer cortex, and epidermis, but with no adventitious roots developed.

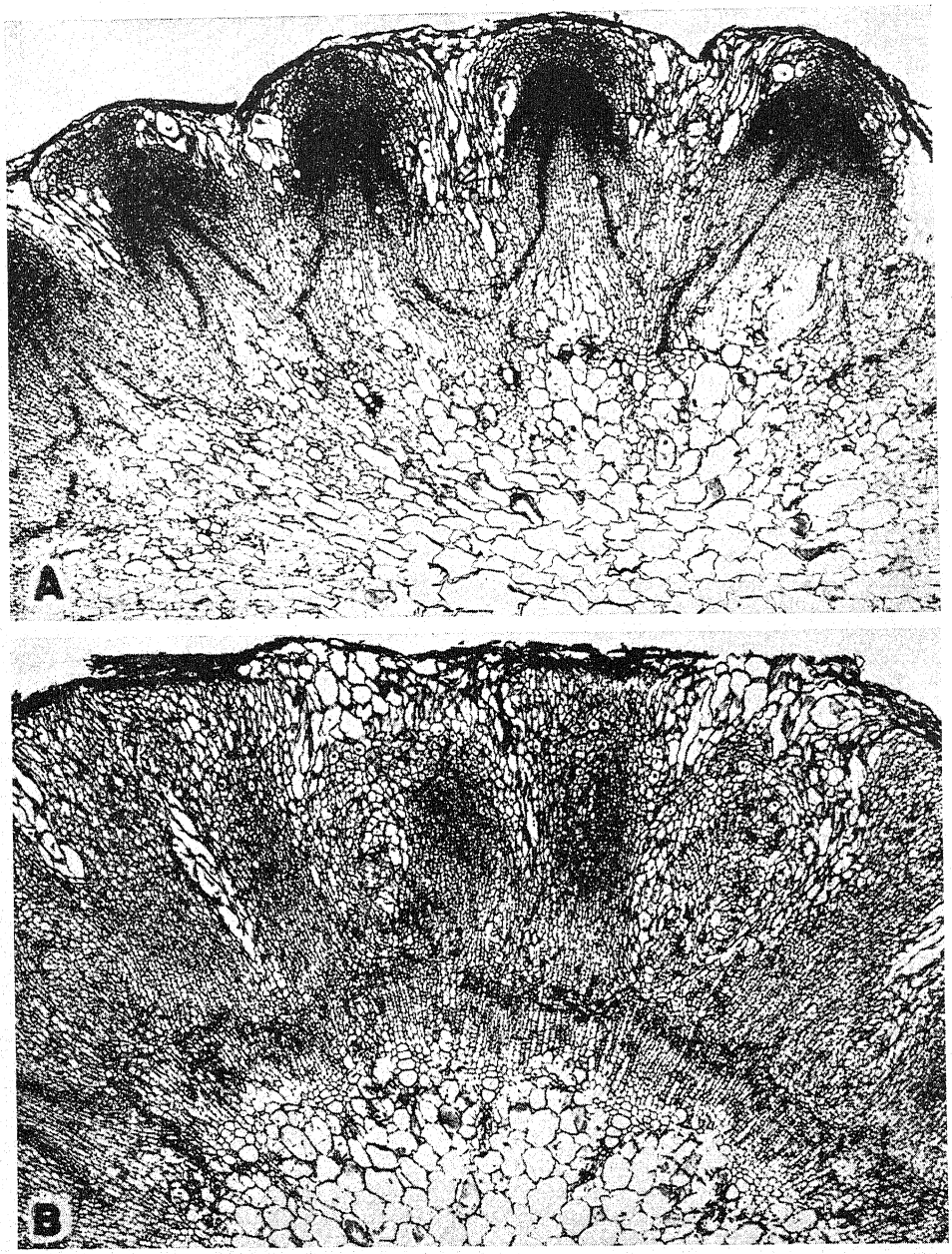


FIG. 14.—Second internode 17 days after apical treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax, showing well-organized adventitious root in each ray. *B*, in lanolin, showing considerable proliferation in ray and phloem parenchyma and smaller amount in cambium but with no definitely organized roots present.

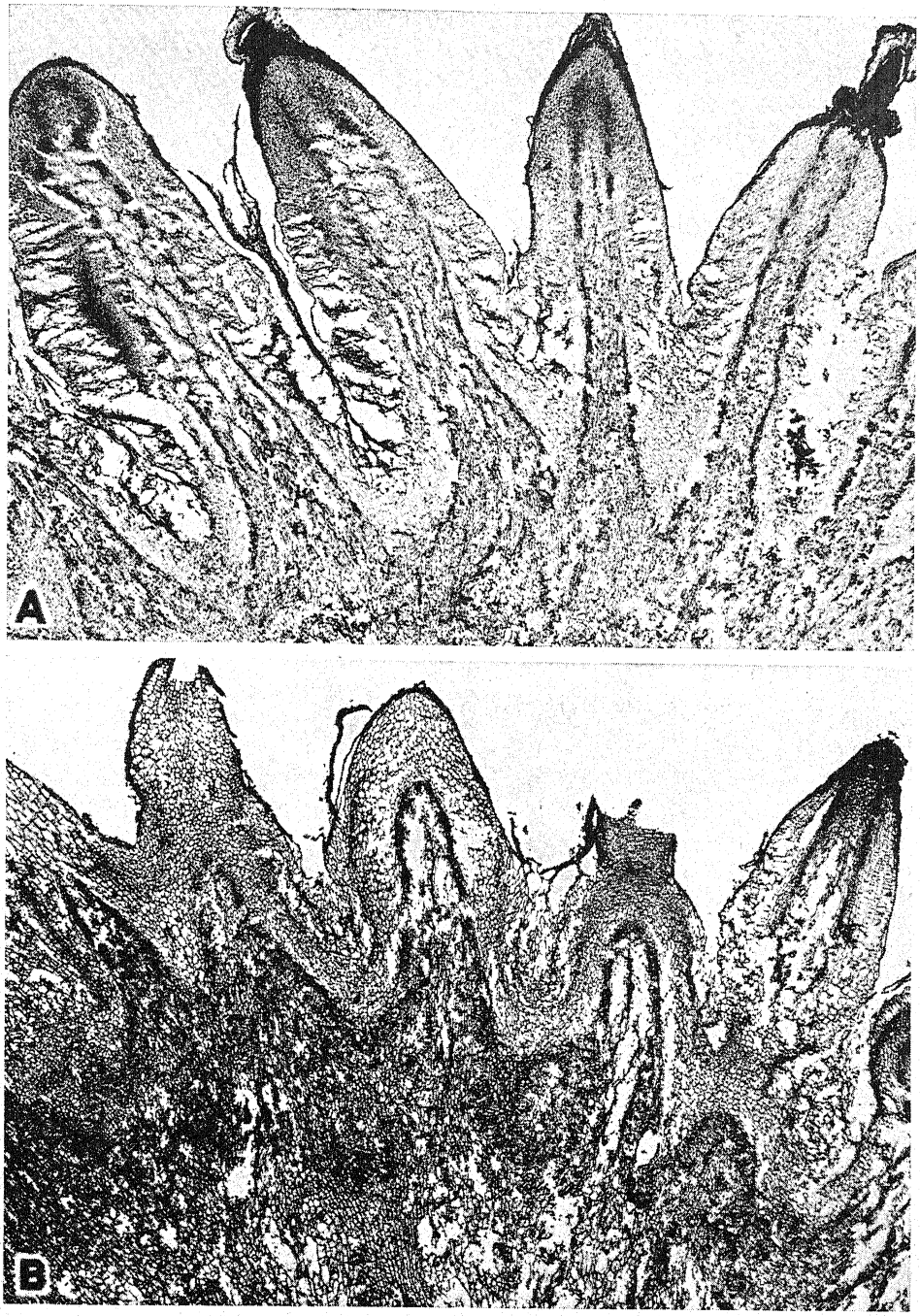


FIG. 15.—Second internode 26 days after apical treatment with 2,4-dichlorophenoxyacetic acid in Carbowax: *A*, at 1%. *B*, at 0.5%. Responses in general are nearly identical; protruded roots are similar, and numerous dead and disorganized areas are evident throughout much of both sections, including disorganized areas in roots also.

thereafter. All the internodes which showed roots protruded from the tumors had had the phenoxy compounds applied to them in Carbowax (figs. 13*A*, 14*A*, 15*A*, B). Although considerable proliferation of various tissues had occurred in the stems of the plants to which the compounds had been applied in lanolin, well-organized roots were usually not induced, and none was ever extruded through the surface of the galls in these experiments (figs. 12*B*, 13*B*, 14*B*).

About 3 weeks after treatment the induced apical tumors began to dry and die, showing slight shrinkage and wrinkling. There were no observable differences between those resulting from 0.5% and 1% mixtures of the substances employed. Transections of tumors at 26 days following application of 1% and 0.5% 2,4-D in Carbowax (fig. 15*A*, *B*) revealed that almost identical responses had resulted and that both tumors were now beginning to die. Epidermis and cortex were completely dead and crushed, the core and outer portions of induced roots showed dead and disintegrating tissues, and there were numerous localized areas in the phloem, rays, xylem, and pith showing dead and disorganized cells. In a few days the tumors became dry and spongy, but none of the plants of these experiments was killed as a result of the application of any of these phenoxy compounds, as had so generally resulted when 2,4-D and 2,4,5-trichlorophenoxyacetid acid had been applied to the bases of the heart-shaped leaves (1).

Discussion

Responses to certain of the substituted phenoxy compounds in both Carbowax and lanolin when applied to decapitated second internodes of the bean were rather strikingly different from those which resulted when the same mixtures were applied to the bases of the heart-shaped leaves (1). Telemorphic effects were practically absent in the decapitated plants. Generally neither stem curvature nor epinasty was shown by these plants, and the responses were chiefly limited to the upper 10–12 mm. of the treated internodes and to the axillary shoots and their leaves, except in those plants on which the Carbowax mixtures melted and ran down along the surface of the first internode and hypocotyl. Even in these cases the responses were also less than in the plants to which these substances had been applied to the heart-shaped leaves. Although the decapitated plants were older at the time of treatment, and hence not so highly meristematic, this of itself scarcely seems sufficient to account for all the differences in responses. A factor of equal, or perhaps of even greater, significance may be associated with the translocation of organic food materials from active photosynthetic regions, as indicated by the studies of MITCHELL and BROWN (7). Little photosynthesis would occur in the decapitated second internode, and there would be comparatively little basipetal movement. Although this internode had comparatively few mature cells in it at the time of decapitation and treatment, the responses were nevertheless generally restricted to the region near the distal end, except in those instances when the Carbowax mixtures melted and ran down the stems. In most of the latter cases the responses were greater, especially in the first internode and the hypocotyl, indicating that there may have been an association between the movement of the growth-regulating substance and the translocation of organic food materials from the heart-shaped leaves attached at the distal end of the first internode. Light and temperature were apparently not differential factors

of significance in these experiments, since all plants of any set of experiments were grown under as nearly identical environmental conditions as could be managed.

Summary

1. Decapitated second internodes of the Red Kidney bean were treated with four of the substituted phenoxy compounds at 0.5% and 1% concentrations, using both Carbowax 1500 and lanolin as carriers. The gross responses were limited largely to the region at, and immediately below, the place of application of the mixtures and to the axillary shoots and their leaves, except when the Carbowax mixtures melted and ran down the plant axis, often over portions of the first internode and sometimes the upper portion of the hypocotyl. Swelling and often root development occurred under these areas. Development of axillary shoots was generally retarded, and frequently their leaves were dwarfed, mottled, or otherwise deformed except following 2,4,5-trichlorophenoxyacetic acid. Application of 2-chlorophenoxyacetic acid induced less response than any of the others. None of the compounds produced epinasty or stem curvature in these experiments, and none of the plants was killed as a consequence of the treatment.

2. Studies of the histological responses were confined to the second internode. The early responses were most evident when lanolin was the carrier, but at 6 days and subsequently the Carbowax mixtures induced greater responses.

3. Responses occurred chiefly in the endodermis, cambium, phloem, and ray parenchyma. The inner cortical cells and the pith were responsive in some stems, especially following application of 2,4-dichlorophenoxyacetic acid in Carbowax. Epidermis, outer cortex, and pericycle were generally unresponsive to all the mixtures.

4. Although some roots may develop internal to the cambium following treatment with 4-chloro- and 2,4-dichlorophenoxyacetic acids, no well-formed roots external to the cambium were found following application of 2- or of 4-chlorophenoxyacetic acids on any plants. Well-formed protruded roots were found only following treatment with 2,4-di- and 2,4,5-trichlorophenoxyacetic acids in Carbowax.

5. Differences in responses between the plants in this experiment and those in which the growth-regulators were applied to the bases of the heart-shaped leaves may in part be associated with greater age of the decapitated plants when treated. A factor of much greater possible significance, however, may be the association of transport of the growth-regulating substances with the movement of organic foods. In the decapitated plants little movement of organic foods occurs in the decapitated second internode and little movement of the growth substance. This may possibly account for the limited telemorphic effects in the decapitated plants as compared with the pronounced responses when the same substituted phenoxy compounds were applied to the bases of the heart-shaped leaves.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BEAL, J. M. Histological reactions of bean plants to certain of the substituted phenoxy compounds. BOT. GAZ. 107:200–217. 1945.
2. BORTHWICK, H. A.; HAMNER, K. C.; and PARKER, M. W. Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. BOT. GAZ. 98:491–519. 1937.
3. HAMNER, K. C. Histological responses of *Mirabilis jalapa* to indoleacetic acid. BOT. GAZ. 99: 912–954. 1938.

4. HAMNER, K. C., and KRAUS, E. J. Histological reactions of bean plants to growth promoting substances. BOT. GAZ. 98:735–807. 1936.
5. HARRISON, B. F. Histological responses of *Iresine lindenii* to indoleacetic acid. BOT. GAZ. 99:301–338. 1937.
6. KRAUS, E. J.; BROWN, NELLIE A.; and HAMNER, K. C. Histological responses of bean plants to indoleacetic acid. BOT. GAZ. 98:370–420. 1936.
7. MITCHELL, J. W., and BROWN, J. W. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. BOT. GAZ. 107:393–407. 1946.

REMOVAL OF 2,4-DICHLOROPHENOXYACETIC ACID AND ITS CALCIUM SALT FROM SIX DIFFERENT SOILS BY LEACHING[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 578

ROBERT W. HANKS

Introduction

Most reports to date on the use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a herbicide deal with spray application to vegetation. The selective nature of the substance has been well demonstrated. The direct treatment of soils with 2,4-D in order to kill weed seeds and for other purposes is chiefly in the experimental stage. Published results of such experiments suggest that the length of persistence of the toxic effect is affected by soil type and management practice. There seems to be considerable variation in persistence of 2,4-D in soils: from less than 4 weeks in muck (3) to as much as 18 months in soil stored in a relatively dry condition (5). The response of clover indicated a difference in the persistence of the acid in Rothamsted clay and Woburn sand (6). KRIES (4) found a longer period of toxicity in soil to which lime had been added a few days prior to application of the acid than in unlimed soil; she noted, however, that additions of partially decomposed leaf mold at the time of liming tended to reduce the degree and persistence of toxicity in the limed soil. BALL (1) reported that toxic effects of the acid were more pronounced in one of three soils tested than in the others, and for a longer period of time. 2,4-Dichlorophenoxyacetic acid was more readily leached from soil than were other herbicides (2), although it is still unknown whether the disappearance of the toxic effect is due chiefly to leaching, to biological decomposition, to a chemical or a physical combination with components of the soil, or to all these causes.

The purpose of the experiment here reported was to determine the relative rates at which 2,4-dichlorophenoxyacetic acid and its calcium salt could be leached from soils possessing different physical and chemical properties. The calcium salt was used in order to determine if it would be more persistent or less readily leached than the acid; this possibility was indicated by KRIES's results (4) on limed soils. Acid and alkaline soils were tested in order to investigate further a possible relationship between soil reaction and persistence or degree of toxicity of the herbicides.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Methods

The sources and some properties of the six soils which were tested are listed in table 1. Soil pH was determined with the glass electrode, moisture equivalent by the centrifuge method.

Each soil lot was thoroughly air-dried and passed through a $\frac{1}{4}$-inch screen in order to obtain a uniform maximum size of soil particles. A small aliquot of each soil was then removed to serve as a control, receiving no chemical treatment. With the exception of soil F, half of each soil was treated with lime ($CaCO_3$) at a rate approximately equivalent to 2000 pounds per acre to a 6-inch depth. Because it was naturally alkaline, only a small aliquot of soil F was limed. The limed aliquots were placed in flats in the greenhouse and were kept moist (not leached) for 2 weeks to permit the reaction of the calcium with other constituents of the soil to approach equilibrium.

TABLE 1

Soil	Source	Physical characterization	pH	Moisture equivalent
A	Beltsville, Md.	Sandy	3.8	10.99
B	Lake Geneva, Wis.	Silt loam	7.7	15.57
C	Lake Geneva, Wis.	Peat	6.7	135.04
D	Chicago, Ill.	Clay loam	8.4	28.55
E	Gary, Ind.	Sandy loam	7.1	15.23
F	Hurley, N.M.	Sandy clay	8.4	25.50

In order to obtain a uniform distribution of 2,4-D and its calcium salt within the soils, sand-herbicide mixtures were prepared quantitatively by pouring an ethyl alcohol solution of the acid and an ethyl alcohol mixture of the salt over pure quartz sand. It was necessary to use the alcoholic mixture of the calcium salt rather than a solution because the salt is insoluble in water, ethyl alcohol, diethyl ether, petroleum ether, acetone, or chloroform. Each batch of treated sand was air-dried and mixed to insure uniformity of this material used in treating the soils.

The sand-acid mixture and the sand-salt mixture were applied to the soils as follows: half of the limed and half of the nonlimed aliquots of each soil were treated with the sand-acid mixture to supply 20 p.p.m. by weight of the acid in the soil; the remaining aliquots of limed and nonlimed soils were treated with the sand-salt mixture to give approximately 22.3 p.p.m. by weight of the calcium salt in the soil. Thus there was applied in equal weights that part of the molecule of these herbicides which is considered to be responsible for their toxic effects (8). Thus, four conditions were obtained for each soil type: (1) lime with the acid, (2) lime with the calcium salt, (3) the acid without lime, and (4) the calcium salt without lime.

After incorporating the sand-herbicide mixtures with the soils, 4-inch glazed crocks with side-hole drains were filled to a depth of approximately 6 inches. Each drain had been covered with glass wool and fitted with a cork stopper and glass tube to facilitate collection of the leachate in Erlenmeyer flasks. There was a minimum of three pots of soil for each treatment of soil A; five or six pots were available for each treatment of the other five soil types.

The potted soils were placed in a basement laboratory in order that water loss by evaporation might be reduced to a minimum and to provide uniform temperature conditions. All pots were wetted initially, with the drains closed, with the equivalent of 4 surface-inches of water. This permitted each soil mass to become thoroughly wetted before any leachate was collected. In addition to the initial 4 surface-inches of water, one-half of the pots in each soil treatment received the equivalent of 10 surface-inches of water over a 6-week period; the other half received the equivalent of 16 surface-inches in the same period. The 10 surface-inches of water were applied at the rate of 1 inch per week for the first 2 weeks and 2 inches per week thereafter. The 16 surface-inches were added at the rate of 2 inches per week for the first 2 weeks and 3 inches per week thereafter. The temperature in the laboratory was 26° C. throughout the experiment.

The leachate from each soil treatment was tested once a week for the presence of herbicide by a slight modification of the bio-assay developed by SWANSON (7). In his method SWANSON used hybrid field corn of the Silver King (Wisconsin no. 7) variety germinated for 48 hours in 6-inch Petri dishes. Twelve to fifteen seedlings with roots between 15 and 25 mm. in length were then placed on filter paper in other 6-inch Petri dishes and moistened with 15 ml. of solution containing 2,4-dichlorophenoxyacetic acid. After 48 hours the increase in average root length of the seedlings in each dish was determined and expressed as a percentage of the increase in average root length of control seedlings treated with distilled water. All germination and growth were in darkness at 26° C. SWANSON determined concentrations of the acid ranging from 1/100 p.p.m. to 2 p.p.m. with only a 5% error.

The bio-assay used in the writer's experiment measured the relative toxicity of the leachates rather than the absolute concentration of the herbicide for which the test was devised originally. SWANSON's method was modified by use of 9-cm. Petri dishes, ten seedlings to the dish treated with 10 ml. of leachate. All other conditions of the original method were unchanged.

Results and discussion

The results of the weekly bio-assays on leachates from soil samples leached with the equivalent of 10 and 16 surface-inches of water showed similar trends within each soil type. Therefore, only the results obtained from the application of the larger amount are reported graphically (fig. 1). The increase in average root length of seedlings grown in leachate from each soil treatment is expressed as a percentage of the increase in root length of seedlings grown in the leachate from untreated control soil of the same type. Each bar of the graph shows this percentage value obtained from the bio-assay of a single leachate. A block of six bars summarizes the results from a single soil treatment, each bar of a block, from left to right, representing the results of each successive week of the 6-week period. Percentage values much greater than 100 may be considered to indicate a stimulation in growth rate caused by the effects of the herbicide; those much below 100 show the relative degree of toxicity of the various leachates.

The results of the bio-assays indicate no significant or consistent difference in the degree of toxicity, in comparable leachates, of the acid and of the calcium salt at the concentrations used. The cal-

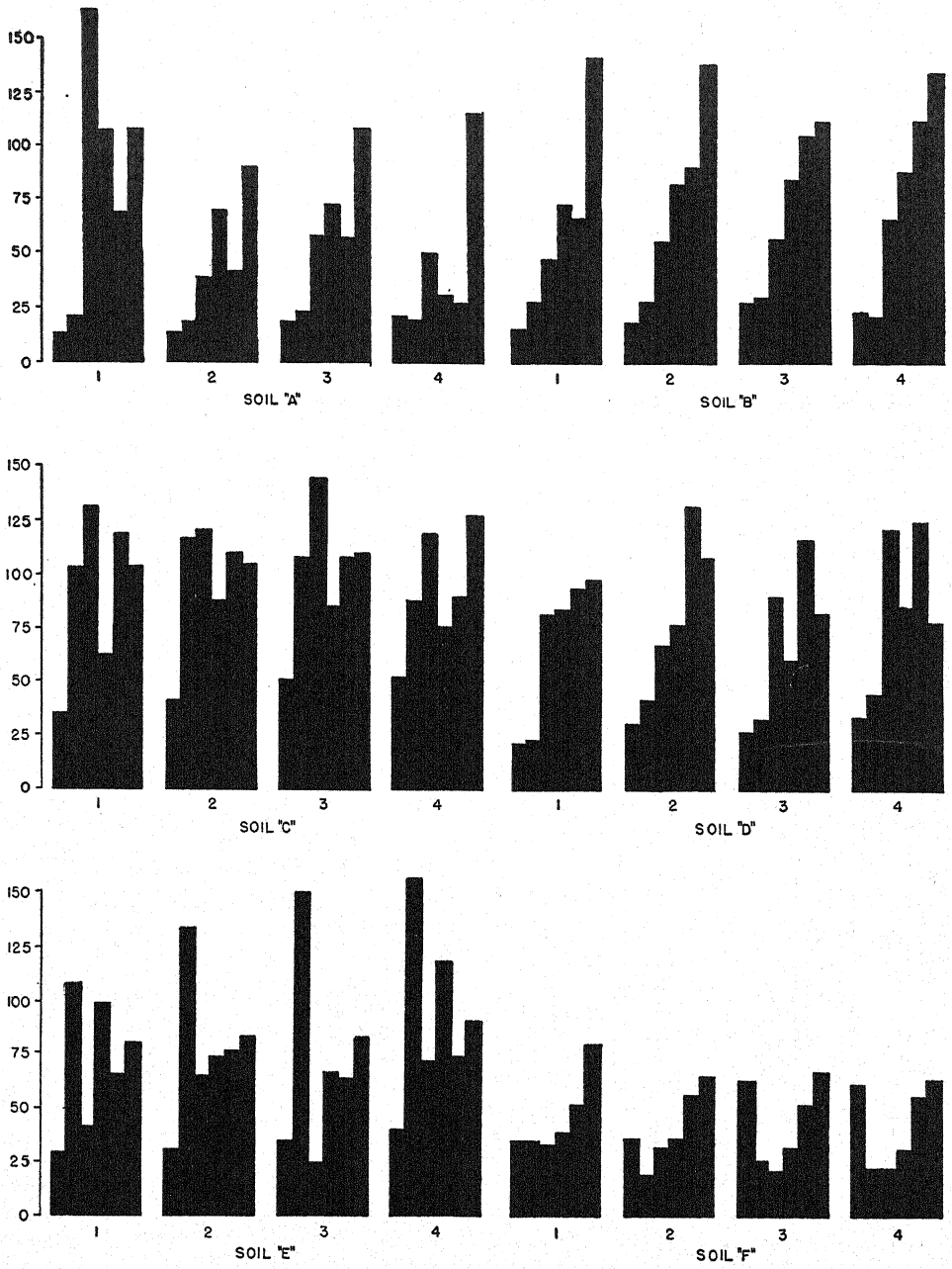


FIG. 1.—Percentage of corn-root elongation in leachate from each soil treatment based on its own control, indicating extent and rate of loss of herbicidal effect. Each bar of a block gives percentage value from bioassay on single leachate. A block of six bars summarizes results from a single soil treatment, each bar from left to right representing results of each successive week of the 6-week period. Numbers below each block designate soil treatments: *1*, limed soil with 2,4-dichlorophenoxyacetic acid; *2*, limed soil with calcium salt of 2,4-D; *3*, nonlimed soil with 2,4-D; *4*, nonlimed soil with calcium salt.

cium salt, or its reaction products, thus seems to be as soluble as the acid in the soil solution of the tested soils, although the calcium salt is insoluble in water, ethyl alcohol, diethyl ether, petroleum ether, acetone, or chloroform. In general, the addition of lime to the soils likewise seemed to produce no significant differences in toxicity of leachates. In contrast, KRIES (4) found a prolongation of toxic effects of 2,4-D in soil in the presence of added lime, although the soil she used was different from those tested by the writer. It may be, also, that the nature of the leachate from soil is such that the degree of sensitivity in the bio-assay is reduced. However, the toxic effects of the herbicides persisted longer in one of the naturally alkaline soils (F) than in any of the other soils tested.

The bio-assay indicated that the leachate from soil C (peat) was relatively free of toxicity at the end of 2 weeks. HAMNER *et al.* (3) reported that the toxic effects of 2,4-D in muck soil disappeared in less than 4 weeks; KRIES (4) found that organic matter added to soil treated with both lime and 2,4-D reduced the period of toxicity. Thus it seems that organic matter in some way reduces the period of toxicity of these herbicides in soil.

The cause of the great fluctuation in the results obtained during the first 3 weeks with soil E is not known. With the fluctuation present in all four treatments of this soil, some unknown, and therefore uncontrolled, factor may have been operating in the control samples of that soil.

The relative toxicity in the successive leachates from soil B decreased at a more uniform rate than in the leachates from the other soils. Soil C was apparently rendered relatively free of toxicity with as little as 4 surface-inches of water applied over a 2-week period, whereas toxic effects were still present in the leachate from soil F after treatment with four times as much water over the 6-week period. Besides leaching, other factors, such as biological decomposition, adsorption by colloidal systems in the soil, and chemical reactions forming nontoxic compounds may be involved in reducing toxicity.

At the end of 6 weeks of leaching treatment, all pots were moved to the greenhouse, and the soil was allowed to dry for 5 days. The soil from each pot was then mixed in such a manner that, when returned to the original pot, the portion that had been at the bottom was uppermost. Thus, any herbicide which had not been removed by leaching from the bottom layers of soil was immediately in contact with germinating seeds. Each pot was planted with ten red kidney beans to determine any residual effects of the herbicide.

This test indicated that soil F was the only soil from which all toxic effects due to the herbicide had not yet disappeared, thus confirming the results of the bio-assay. In this soil, the toxic effects were found to be greater in those pots which had been leached with only 10 surface-inches of water than in those leached with 16 inches. The toxicity did not affect the percentage of germination and was shown chiefly in the malformation and mottled appearance of the primary and first trifoliate leaves.

Summary

1. Six soils with a wide range in pH and in physical properties were treated with lime, 2,4-dichlorophenoxyacetic acid, and the calcium salt of the acid in such a manner as to obtain four conditions for each soil.

2. The treated soils were leached with

water at rates equivalent to 10 and 16 surface-inches over a 6-week period. The leachates were collected and tested weekly with a corn-seed bio-assay to determine relative toxicities.

3. The bio-assays indicated that the leachates from soil C (peat) were relatively nontoxic at the end of 2 weeks and that those from all soils except soil F (naturally alkaline) were relatively free of the herbicides at the end of 6 weeks. There were no significant differences in the degree of toxicity and its persistence in limed as compared with unlimed samples within each soil type or in that of the calcium salt versus the acid.

4. At the end of 6 weeks, the bottom half of each soil mass was tested for residual toxic effects by the germination of red kidney beans. This test indicated that soil F was still somewhat toxic from the treatment and that the samples of this soil leached with 10 surface-inches of water were more toxic than those leached with 16 surface-inches of water. All other soils were then free of the toxic effect of the herbicide.

The author wishes to express gratitude to Dr. CHARLES E. OLMSTED for advice and close interest in the problem.

DEPARTMENT OF BOTANY
UNIVERSITY OF MIAMI
CORAL GABLES, FLORIDA

LITERATURE CITED

1. BALL, W. S. Report on 2,4-D experiments in the western states. Proceedings of the Second Annual Meeting of the North Central States Weed Control Conference. Nov. 26–28, 1945.
2. DE ROSE, H. ROBERT. Persistence of some plant growth-regulators when applied to soil in herbicidal treatments. BOT. GAZ. 107:583–589. 1946.
3. HAMNER, C. L.; MOULTON, J. E.; and TUKEY, H. B. Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid on germination and development of seedlings. BOT. GAZ. 107: 352–361. 1946.
4. KRIES, OLIVE. Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime. Bot. GAZ. 108. 1947. (In press.)
5. MITCHELL, J. W., and MARTH, P. C. Germination of seed in soil containing 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 107:408–416. 1946.
6. NUTMAN, P. S.; THORNTON, H. G.; and QUASTELL, J. H. Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant-growth substances. Nature 155:498–500. 1945.
7. SWANSON, CARL P. A simple bio-assay method for the determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solutions. BOT. GAZ. 107:507–509. 1946.
8. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–343. 1946.

HISTOLOGICAL RESPONSES OF BEAN PLANTS TO NICOTINE AND TO WOUNDING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 579

A. GERALDINE WHITING AND MARY AILEEN MURRAY

Introduction

Nicotine is one of the well-known plant alkaloids, a group of compounds which have been used in many different types of experiments. Some of these alkaloids possess medicinal properties, and many are intensely poisonous, their effect depending in part upon the concentration used. According to MACHT and DAVIS (12), nicotine is very toxic not only for animal but also for living plant protoplasm. They recorded inhibition of root growth of *Lupinus albus* in 1:5,000 solutions of various forms of nicotine. Likewise, when EISLER and PORTHEIM (5) soaked certain seeds in 0.1–0.4% nicotine solutions, tobacco failed to germinate and buckwheat seedlings were injured. In contrast, SONNENSCHEIN (20) found that growth was stimulated when soybean seeds were soaked in a 0.2% solution for 24 hours. He also noted that nicotine in combination with atropine or with colchicine induced polyploidy. NEMEC (17) described certain effects of nicotine upon cell division in root tips of the pea. From experiments with young corn plants grown in nutrient solutions containing nicotine tartrate (*m*/100 to *m*/10,000), WEIS *et al.* (22) reported that nicotine was harmless to the plants.

The application of nicotine sulphate as an insecticide has been a widespread horticultural practice for nearly two centuries. In 1940 MARTIN (13) stated that no record could be found of any damage to plants caused by the nicotine itself in washes and dusts used as insecticides. During greenhouse fumigation experiments with concentrations of nicotine as high as 30–60%, RICHARDSON *et al.* (18) observed no apparent injury to crops of lettuce, tomato, and various other plants. More recently WITHROW and BIEBEL (24) reported that young leaves of Biloxi soybean showed chlorosis after exposure to a 14% nicotine fumigant. However, they suggested that other products in the fumigation process may have been involved.

Experimentation with nicotine as a growth-regulating substance was reported by TRAUB (21) in 1938. He tested over one hundred different compounds for their effectiveness in promoting the initiation of roots in cuttings of several different subtropical plants. In comparison with other substances tested, nicotine in a 0.01% solution was rated in the highest degree of superiority. This placed it in the same category as 0.02% indolebutyric acid, which TRAUB considered the most useful of the compounds tested.

Material and methods

The present report on nicotine is a continuation of the series of experiments (3, 7, 8, 9, 10, 15, 23) on the histological response of bean plants (*Phaseolus vulgaris* var. Red Kidney) to various growth regulating substances. Methods were similar to those described for the earlier experiments. Observations and collections of material were made at various intervals up to 30 days after decapitation. The specimens were preserved in Nava-

shin's solution, imbedded by the butyl alcohol–paraffin method, sectioned at 10–15 μ, and stained in a modified triple stain. The nicotine was obtained from the Eastman Kodak Company.

Four different lots of bean plants were used. Because histological examination of the first lot showed marked similarity between treated and control plants, the original experiment was repeated and extended with greater consideration given to control plants and natural wound response.

LOT I.—These plants were grown in the greenhouses of the Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Beltsville, Maryland, in May and June, 1939. Young plants of uniform size with the first trifoliate leaf unfolding were decapitated near the apex of the lengthening second internode. The treated plants, the greater part of the lot, received an application of 2% nicotine in anhydrous lanolin smeared over the cut surface. Some of the control plants were only decapitated; on others, lanolin alone was applied to the cut surface.

LOT II.—This experiment, in general a repetition of the first, was conducted in the greenhouses of the University of Chicago in May and June, 1946. The number of control plants equaled that of plants treated with nicotine. One half of the latter received an application of 2% nicotine in lanolin; the other half, 3% nicotine in lanolin. Control plants were treated in two ways: simple decapitation and decapitation plus an application of lanolin.

LOT III.—Plants from lot II were used for further experimentation in wound response of control material. In each plant the second internode of the strongest axillary branch, which developed after the removal of the main stem, was decapitated. One half of these branches received no further treatment; the other half, an application of lanolin only.

LOT IV.—This lot of material was derived from an experiment in which bean plants were decapitated in the upper portion of the hypocotyl 7 days after planting. No further treatment was given, and collections were made at various intervals after decapitation. DOUTT (4), in her study of the anatomy of the bean plant, considered the upper portion of the hypocotyl similar in structure to the stem except for the tangential arrangement of the primary xylem (fig. 15*A*).

Observations

GROSS RESPONSES

LOT I.—The response of the two lots of plants treated with nicotine differed considerably in degree but not in kind. In lot I treatment with 2% nicotine induced a strong response, and a well-defined tumor was formed. The following description of gross response is based on observation of this first lot of material in which treatment was more effective.

The gross response of the decapitated stem to 2% nicotine indicates that only the uppermost and peripheral portions of the tip were obviously stimulated by treatment with nicotine. Visible response was initiated slowly. By the 3d day the apex showed a central depression in the pith and a slight flaring of the outer tissues. This outward flare extended down the stem less than 1 mm. By the 5th day the central cavity had deepened and was lined with injured or suberized pith cells. Occasionally, the xylem tissues at the margin of the cavity stood up as a slight ring or crown. Tissues outside this xylem ring were flared outward to a depth of 1.5 mm., and the stem tip had broadened

to twice its original diameter. The outermost tissues were then at right angles to the stem axis and gave the apex the appearance of a flat nailhead with a hollowed center. After the 7th day the stem below the cut surface changed little in appearance.

Proliferation of the flared stem tissues in the formation of the tumor was evident above the original level of the cut surface by the 5th day. The most rapid growth occurred between the 5th and 7th days and was followed by a period of gradual enlargement. In later stages the rate of growth appeared to diminish, but increase in size was noted until observations were discontinued at the end of 30 days. At this time a large tumor measured 13 mm. in transverse diameter and 8 mm. in length. In early stages of growth the proliferation was pale in color. Within a few days the tumor became light green, succulent in appearance, and finely tuberculate over the surface. Later growth was accomplished by maturation, and older tumors appeared firm and compact. Portions of the surface were brown where cells were suberized. In shape the tumor was flat across the top with the peripheral portions growing downward and closely encircling the stem (figs. 8*B*, 9*A*). These marginal areas showed the greatest growth and, in later stages, formed the larger part of the tumor. The central cavity was a characteristic feature and became partially filled or overgrown by proliferating tissues in older stages. The tumor was generally symmetrical as compared with the irregular tumors induced by such substances as indoleacetic acid. At no time was there evidence of the presence of root primordia.

Only brief observations were made of control plants grown in lot I. Decapitated stem tips, receiving no further treatment, occasionally developed small tumors. Those which received an application of lanolin showed consistently greater response, although no large tumors were formed. There was irregularity in the form of these tumors and in the degree of response. In one plant treated with lanolin a tumor which formed within 7 days after treatment was larger than some formed during 20 days' growth in other instances.

LOT II.—In this lot plants treated with 2% nicotine failed to show the vigor of response of the earlier experiment, and small irregular tumors were formed. Plants treated with 3% nicotine more nearly reached the degree of response evident in lot I.

Since particular emphasis was placed on control plants in lot II, observations of gross responses were more complete for this group. However, conditions for growth were apparently less favorable than with lot I. Simple decapitation of the second internode rarely induced tumor formation. The cut surface became brown and dry, and some shrinkage of the tissues occurred at the apex. Below the apex the tissues remained firm and green for a period of 3–4 weeks after decapitation. Following this period, yellowing became apparent in many stem tips, and they died back $\frac{1}{2}$ inch or more. In the lanolin-treated plants paling of the surface was marked, but the surface itself remained fresh in appearance. Generally a slight flaring of the tissues was detectable at the cut surface by the end of the 3d day. Further development was unpredictable. Often no more proliferation or callus formation was evident. In some plants tumors developed in 7–12 days (fig. 11*A*); in other plants a similar size was not attained until 27–31 days (fig. 11*B*). These tumors were irregular in form, and frequently only one sector of

the stem tip was involved (fig. 11*C*). No large tumors were observed.

LOT III.—In this lot the decapitated second internode of the axillary branch received treatment comparable with control material in the first two lots. However, the diameter of the axillary branch was greater and the tissues appeared to be more mature; the response was likewise greater in degree. Branches which were simply decapitated showed a darkening and drying of the surface; in those which received an application of lanolin, the cut surface appeared fresh and succulent under this covering. In both cases slight yellowing and flaring of the stem tip were detectable by the 2d day. Enlargement continued, and by the 5th–7th days it was clearly evident that the region of growth was a band of proliferating tissue lying between the outer tissues and the xylem. The xylem appeared as a distinctive ring or crown of darkened tissue, and the center of the stem was somewhat sunken and dried. Further growth changed the pattern but little. The band of proliferating tissue increased in width to form a broadly flared tumor which abruptly tapered downward to the diameter of the unaffected stem. Such tumors might reach a width of 6–7 mm. across and 4–5 mm. in length at the end of 2–3 weeks. There was no growth of peripheral portions of the tumor downward to encircle the stem, such as was noted for nicotine treatment.

LOT IV.—The response in this lot, composed of decapitated hypocotyls, was similar to that of the axillary branch of lot III. The broadly flared stem tip showed the band of proliferating tissues, the protruding crown of matured xylem elements, and a central cavity resulting from the disintegration of pith.

HISTOLOGICAL RESPONSES

NICOTINE.—The following detailed description of the histological responses of bean to nicotine is based upon a study of the first lot of plants. Nicotine, as applied in this experiment, induced responses only in the uppermost levels of the stem. The greatest proliferation occurred within 1 mm. of the surface of application; below this level the response diminished in degree and extended beyond 2–3 mm. only in the endodermis and in the cambium. Response varied from plant to plant, but these differences were more evident in the rate of reaction than in the pattern of response.

The epidermis and the cortical parenchyma were frequently injured at the cut surface and were limited in their response. Epidermal cells immediately below the injured ones might enlarge slightly and by the 3d day undergo one or two transverse divisions (fig. 1*A*). Further response consisted of the usual growth to allow for expansion of internal tissues. The downward extent of response in the cortical parenchyma was generally less than 0.5 mm. below the cut surface. Enlargement and proliferation of the cells were evident by the 3d day (fig. 1*A*) and continued through the 5th day. The degree of proliferation varied considerably from none at all to divisions in various planes in the upper cells (fig. 2). These divisions did not, however, contribute to the proliferation of a tumor above the cut surface.

The endodermis was more sensitive than the cortical parenchyma. By the 3d day (fig. 1*A*, *C*) endodermal cells down to about 1 mm. were enlarged, and proliferation was initiated in the uppermost cells. Maximum development was reached by the 5th day. Because of the outward flaring of tissues at the stem

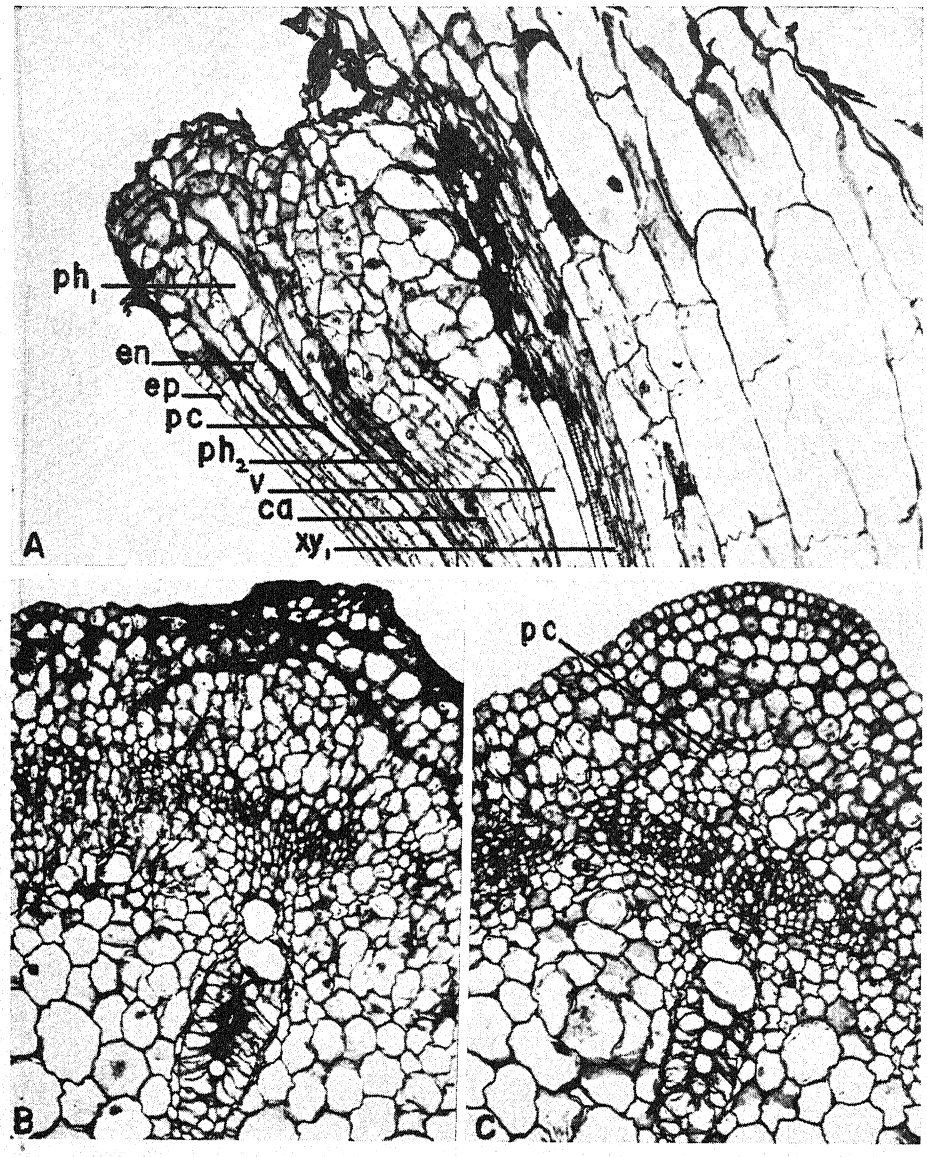


FIG. 1.—Decapitated second internodes, 3 days after treatment with 2% nicotine in lanolin. *A*, longisection: *ep*, epidermis; *en*, endodermis; *pc*, pericycle; ph_1, primary phloem; ph_2, secondary phloem; *ca*, cambium; *v*, vessel; xy_1, primary xylem. Initial proliferation of cells of endodermis, phloem parenchyma, and meristematic zone (cambium and its undifferentiated derivatives); a few divisions in epidermis; pericycle crushed; pith inactive. *B*, transection, 0.3 mm. below cut surface. Epidermis and cortex injured; pericycle over bundle crushed; parenchymatous cells of phloem and rays enlarged. Note characteristic elongation of xylem parenchyma encircling mature vessels. *C*, same stem as *B*, 0.5 mm. below cut surface. Endodermal cells enlarged radially. (Material for figs. 1–9 taken from lot I.)

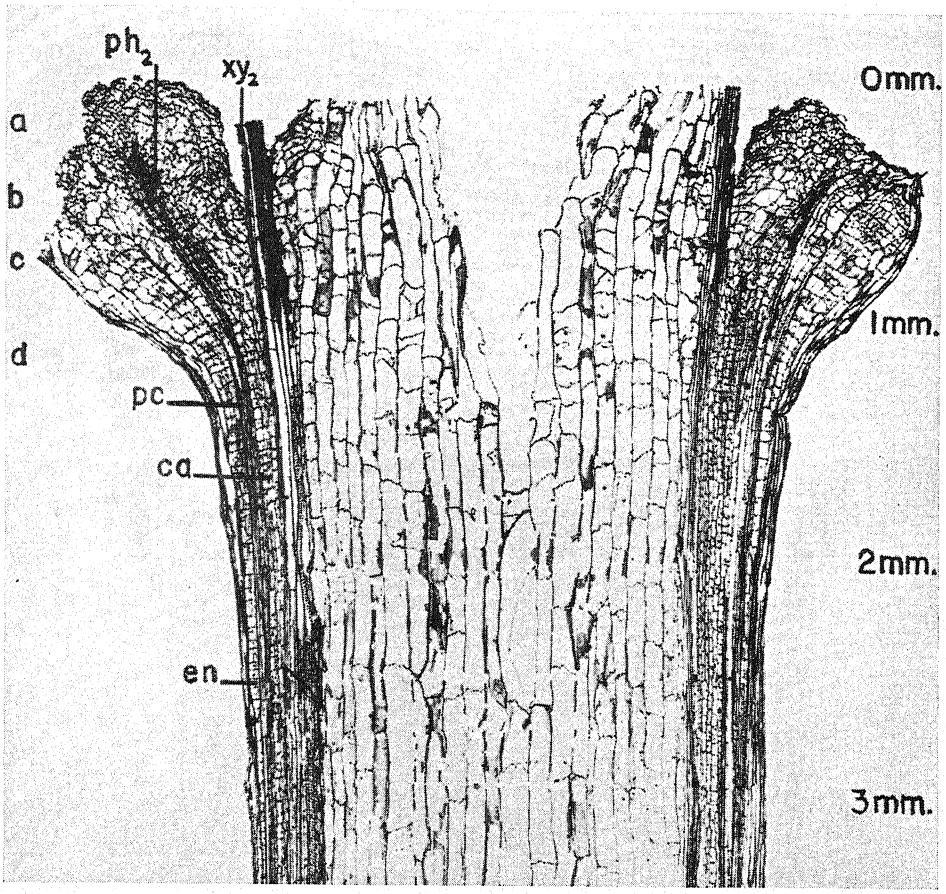


FIG. 2.—Longisection, 5 days after treatment with nicotine: xy_2, secondary xylem; *o mm.*, approximate level of cut surface as determined by severed vessels, outer tissues having been deflected downward. At apex proliferation of cortical parenchyma, endodermis, phloem parenchyma, and meristematic zone. Below cut surface enlargement and division of endodermal cells; pericycle crushed; secondary phloem sieve tubes and companion cells recognizable within short distance of cut surface. Meristematic zone very active and forming wedge-shaped area of proliferated tissue, which is torn from inactive secondary xylem at stem tip. Central pith disintegrating. Levels *a*, *b*, *c*, *d* similar to transections in figs. 3 and 4.

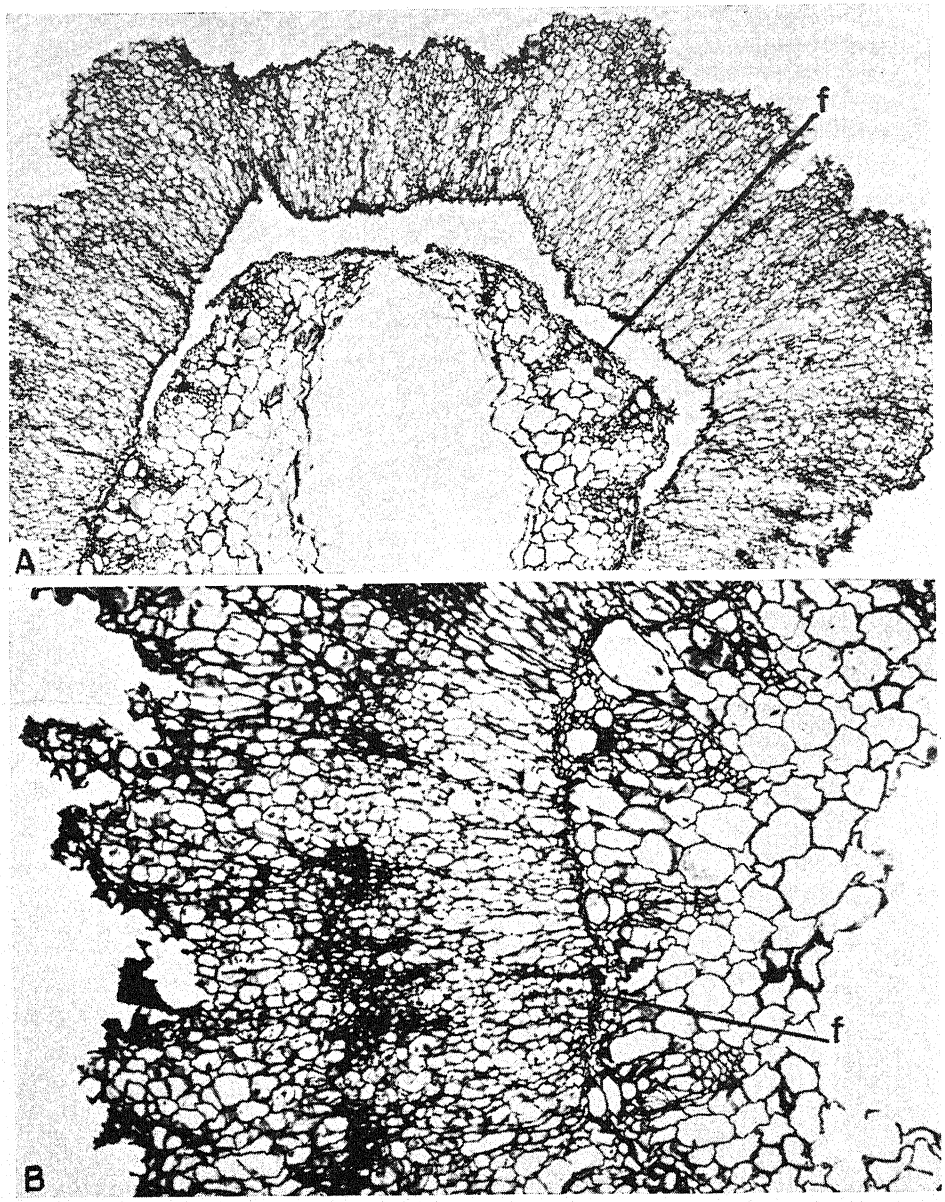


FIG. 3.—Transections, 5 days after treatment with nicotine. *A*, just below surface of application: *f*, secondary xylem layer of fibers. Outer segment proliferated mainly from meristematic zone; inner segment bordered on outer margin by fiber layer (*f*) and composed of inactive xylem, inner portion of ray, and pith tissues. Compare with level *a*, fig. 2. *B*, same stem, 0.5 mm. below cut surface. Marginal proliferated tissue derived from primary phloem parenchyma (tissues from pericycle outward deflected below this level); secondary phloem sieve tubes appearing as dark patches; wide band of proliferated tissue in meristematic zone; layer of secondary xylem fibers differentiated around stem; cells of inner portion of ray and xylem parenchyma adjacent to matured vessels much enlarged; central pith disintegrating. Compare with level *b*, fig. 2.

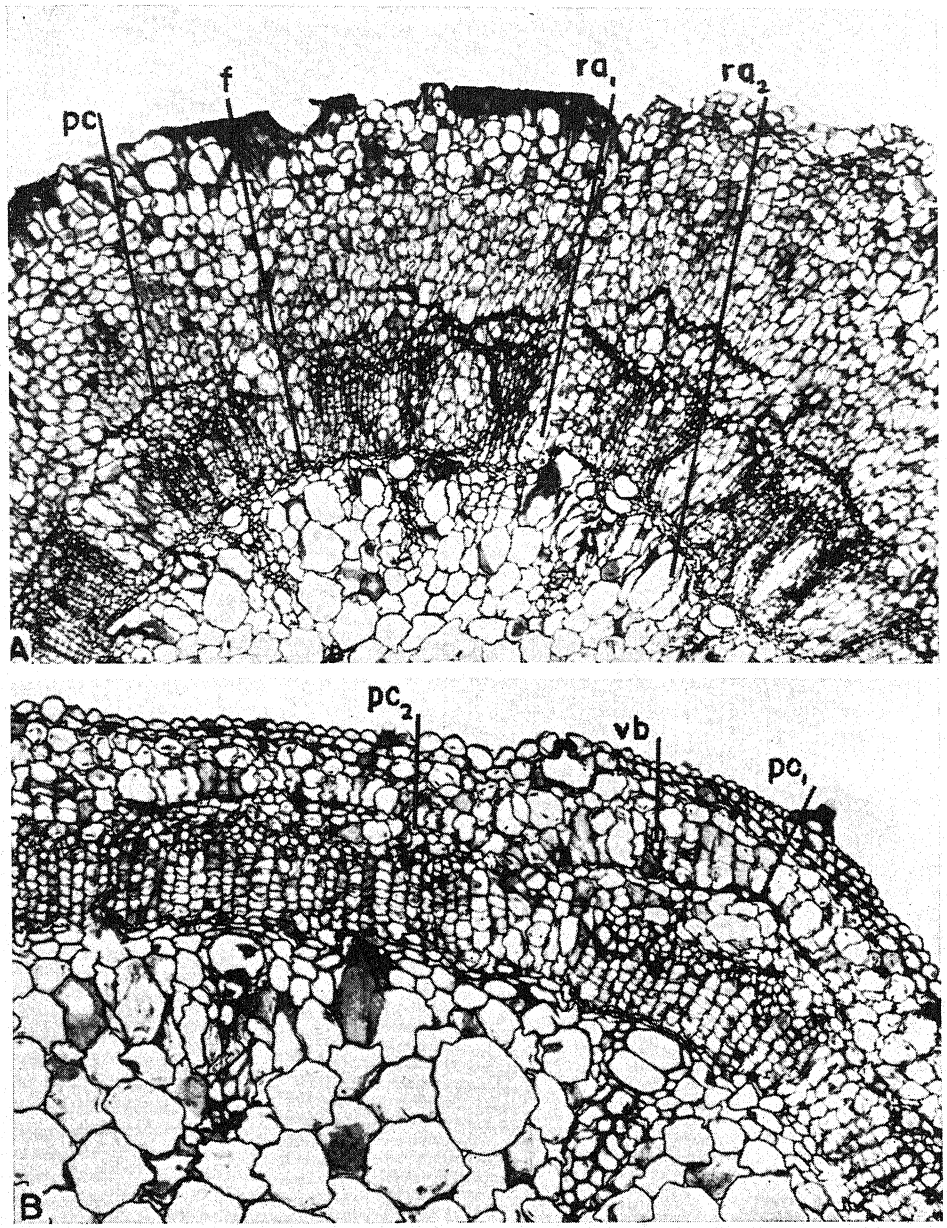


FIG. 4.—Transections, 5 days after treatment, same stem as fig. 3. *A*, 1 mm. below cut surface: *ra*, ray. Outermost cells enlarged cortical parenchyma; endodermal derivatives forming broad band because of outward deflection at stem tip; pericycle conspicuously crushed over bundles; wide band of proliferating cells in meristematic zone; outer portion of ray (ra_1) keeping pace with meristematic zone by means of cell enlargement and a few divisions; many cells of inner portion of ray (ra_2) greatly enlarged; layer of fibers interrupted across some rays. Compare with level *c*, fig. 2. *B*, same stem, 1.5 mm. below cut surface. Endodermal cells enlarged radially and a few divided tangentially; initiation of vascular bundle (*vb*) from derivatives of endodermis, occurrence infrequent. Pericyclic cells crushed (pc_1) or maturing as fibers (pc_2). Meristematic zone not so wide as at higher levels. Compare with level *d*, fig. 2.

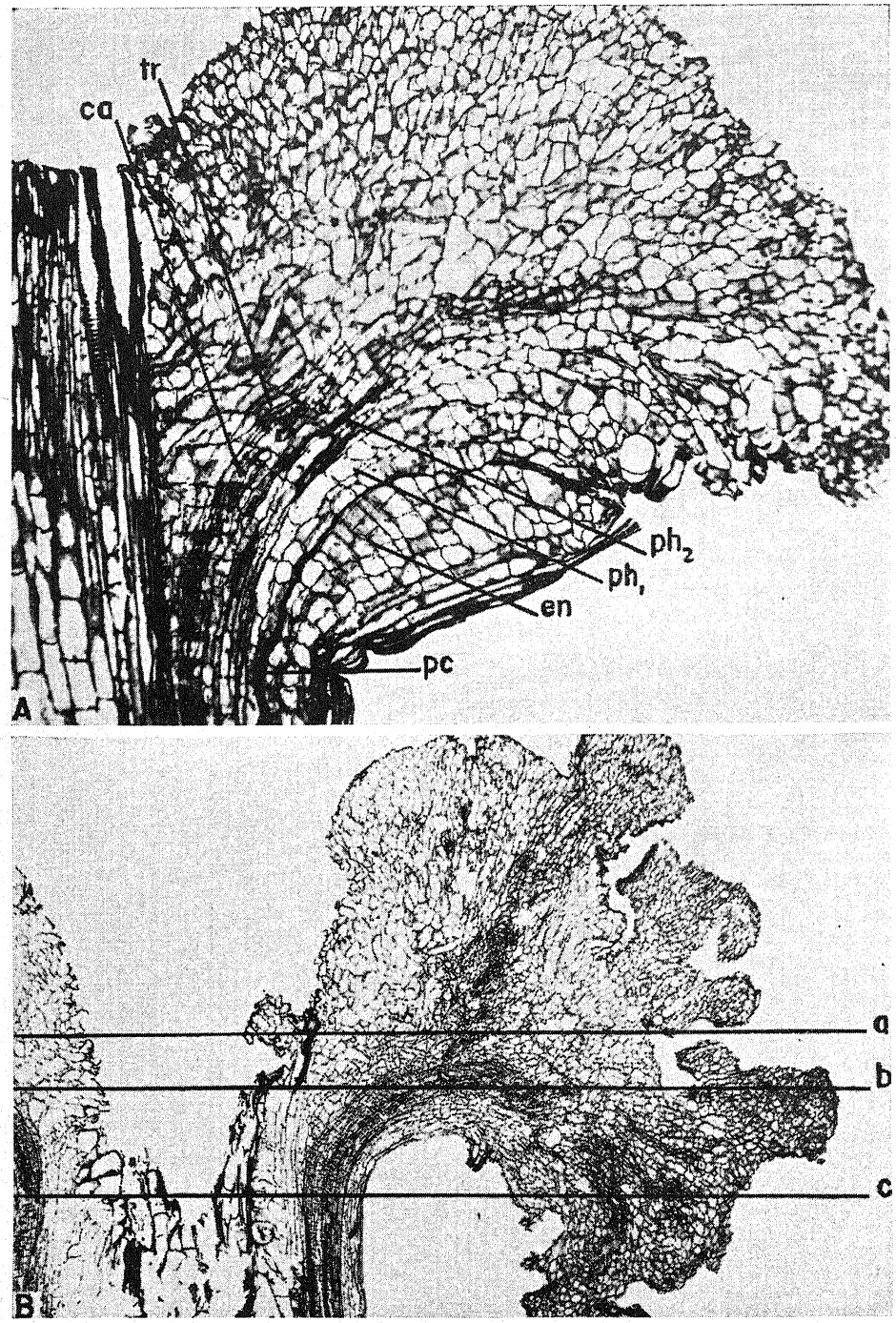


FIG. 5.—Longisections of decapitated stem treated with nicotine. *A*, 7 days: *tr*, tracheid. Early stage in development of tumor derived mainly from upward proliferation of meristematic zone; outermost tissues cut off by crushed pericycle. Beginning of maturation in derivatives of meristematic zone evident in differentiation of tracheids (*tr*) immediately centrad to cambium (*ca*). *B*, 16 days. More mature stage in development of tumor, showing layer of tracheids derived from cambium below original level of treated surface. Differentiation of vascular strands in tissue proliferated above treated surface. Levels *a*, *b*, *c* corresponding approximately to transections in figs. 6*A*, *B*, 8*B*, respectively.

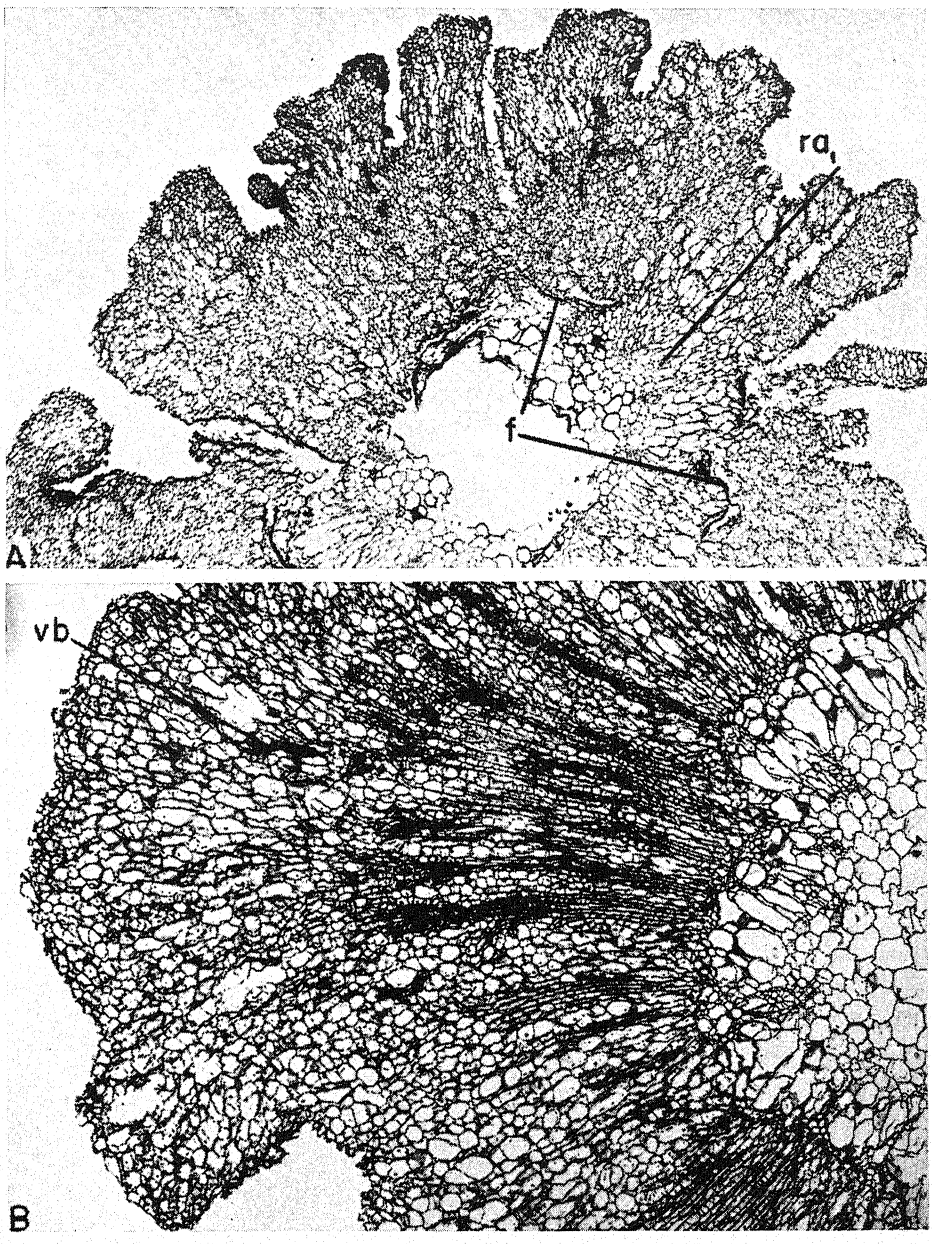


FIG. 6.—Transections of stem, 12 days after treatment with nicotine. *A*, at cut surface. Outer region highly meristematic periphery of tumor. Enlargement and slight proliferation of tissues centrad to secondary xylem layer of fibers (*f*); greater activity in rays lacking fiber layer (ra_1). Compare with level *a*, fig. 5*B*. *B*, same stem, 0.5 mm. below cut surface. Pith inactive. Parenchyma cells of xylem and ray centrad to fiber layer responding mainly with enlargement. Outside fiber layer radial rows of cells are vascular tissue differentiated from meristematic zone and deflected to horizontal position. Cells in remaining (outer) portion of tumor maturing generally as parenchyma; some continuing activity to form vascular bundles (*vb*); others enlarged, divided, and collapsed; a few at periphery remaining meristematic. Compare with level *b*, fig. 5*B*.

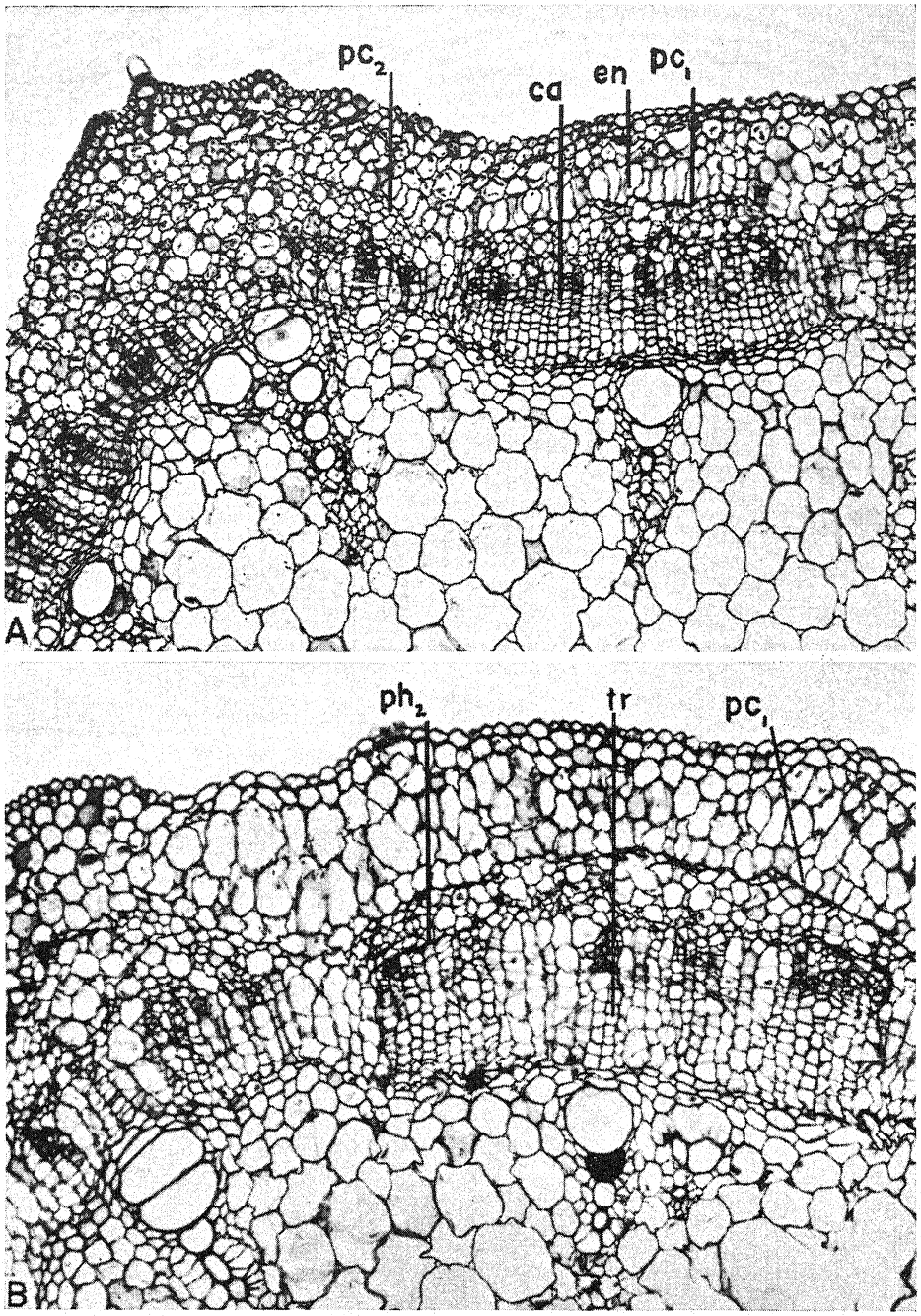


FIG. 7.—Transections of same stem as fig. 6, 12 days after treatment. *A*, 1 mm. below cut surface. Endodermal cells enlarged radially and a few dividing; many pericyclic cells crushed (pc_1) but some maturing into fibers (pc_2). Derivatives of meristematic zone differentiated as phloem and as xylem tracheids; narrow meristematic layer including cambium (*ca*); ray cells maturing as parenchyma. *B*, same stem, 1.5 mm. below cut surface. Response similar to that at 1 mm. Secondary phloem which matured before treatment is crushed (ph_2). Compare with transection of stem in fig. 8*B*.

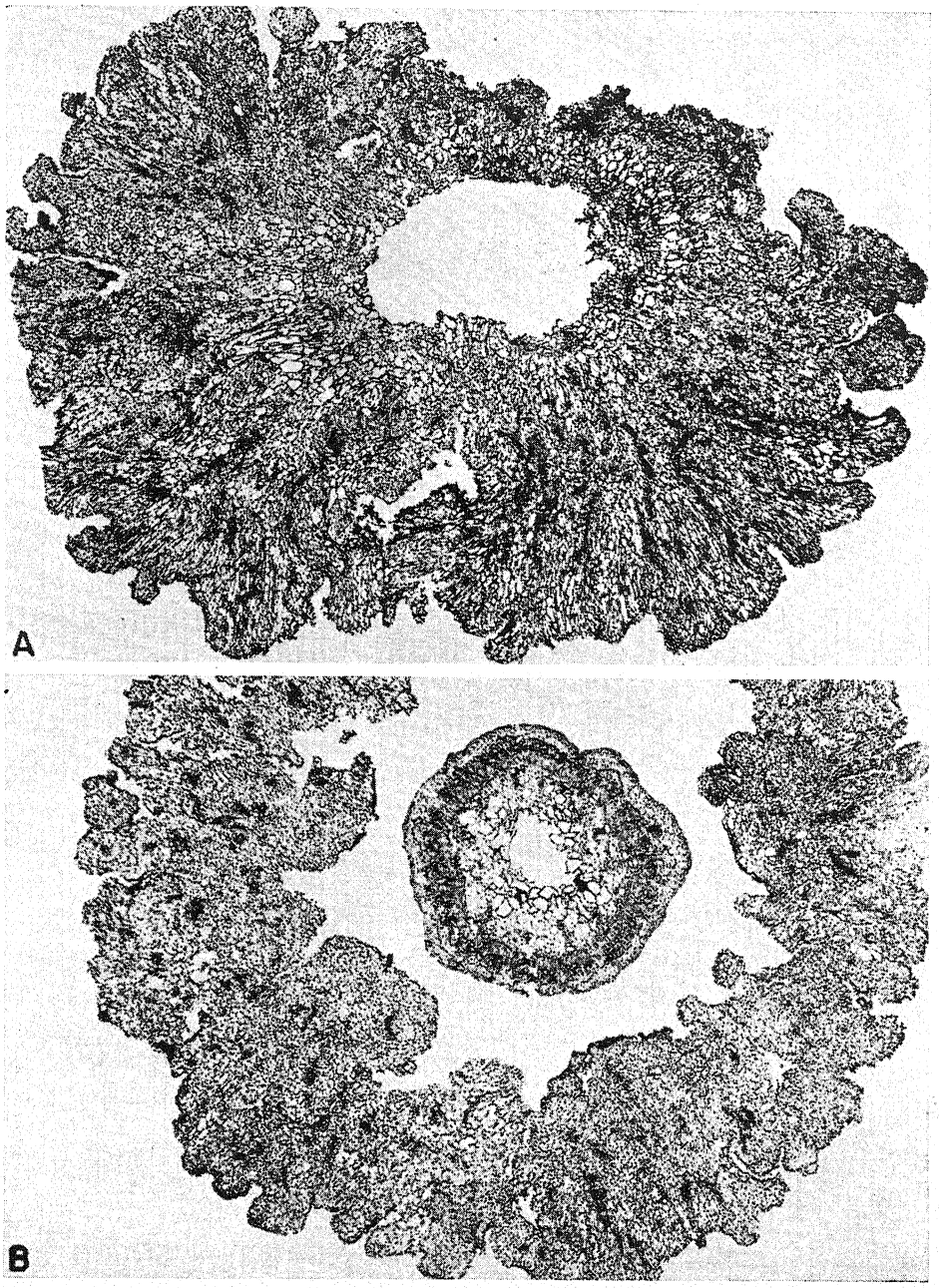


FIG. 8.—Transections of tumors, 19 days after treatment with nicotine. *A*, immediately below cut surface. Irregular proliferation at periphery results in tuberculate surface. Vascularization more advanced than in fig. 6*B*. *B*, 1.5 mm. below cut surface. Overhanging portions of tumor closely formed about stem axis in center. Compare with level *c*, fig. 5*B*. Tumor parenchymatous and highly vascularized (dark patches). Response in stem evident in wide band of tissues differentiated from meristematic zone.

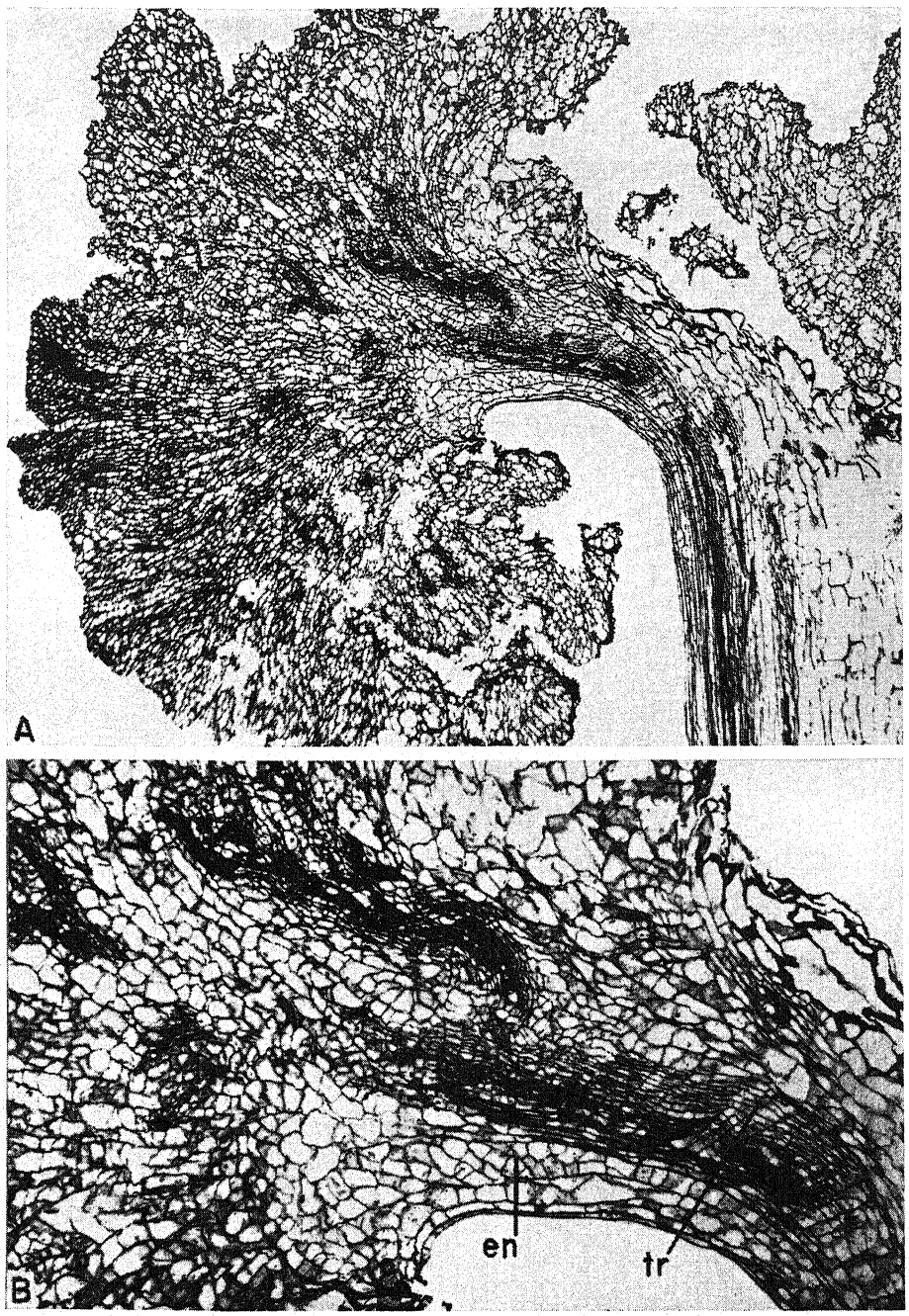


FIG. 9.—Longisection of apical tumor, 21 days after treatment with nicotine. *A*, growth of tumor in outward and downward directions; central cavity partially filled by overgrowth. *B*, detail of *A*. Vascular complex of tumor directly connected with vascular tissues in stem. Note general maturation of proliferated tissues as parenchyma, except for vascular bundle originally differentiated from meristematic zone (see fig. 5*A*) and now considerably enlarged through activity of cambium.

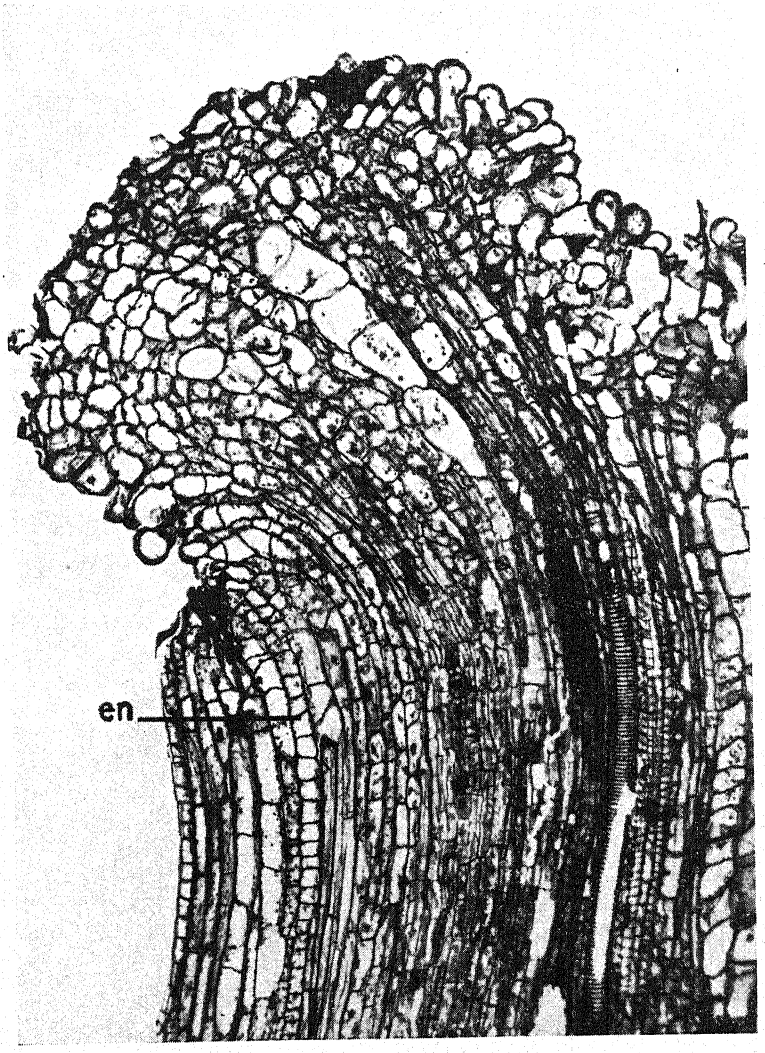


FIG. 10.—Decapitated stem from lot I, 20 days after treatment with lanolin only. At cut surface epidermis and cortical parenchyma injured; enlargement and slight proliferation of endodermal and pericyclic cells. Phloem parenchyma enlarged and proliferated; marked activity in cambium and adjacent undifferentiated cells (meristematic zone); segments of immature xylem vessel conspicuously enlarged. Superficial tumor formed mainly from surface proliferation of phloem, meristematic zone, and unlignified xylem tissues. Transverse phellogen forming in pith.

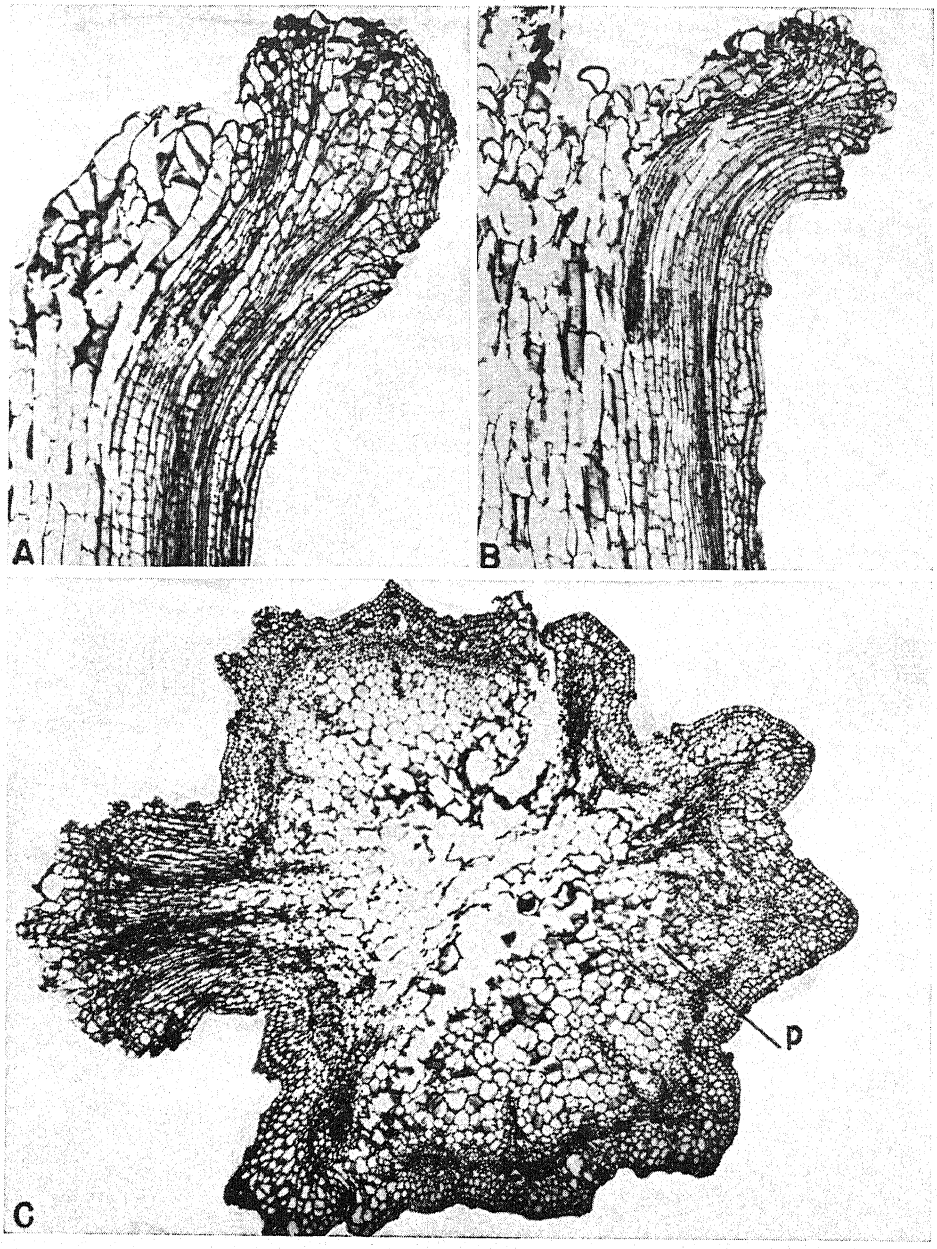


FIG. 11.—Decapitated stems from lot II, treated with lanolin only. *A*, longisection, 9 days. Development of superficial tumor similar to fig. 10. Transverse phellogen lacking. *B*, longisection, 27 days. Although considerably older, tumor similar to fig. 11*A* in extent of development. *C*, transection about 0.5 mm. below cut surface, 27 days: *p*, phellogen. Inequality of response over cut surface shown in proliferation of small callus at left, evidence of collapse of tissues at upper right, and formation of phellogen (*p*) in lower right sector. Note relatively immature stage in development of stem tissues and absence of secondary xylem fiber layer, characteristic of material in lot II.

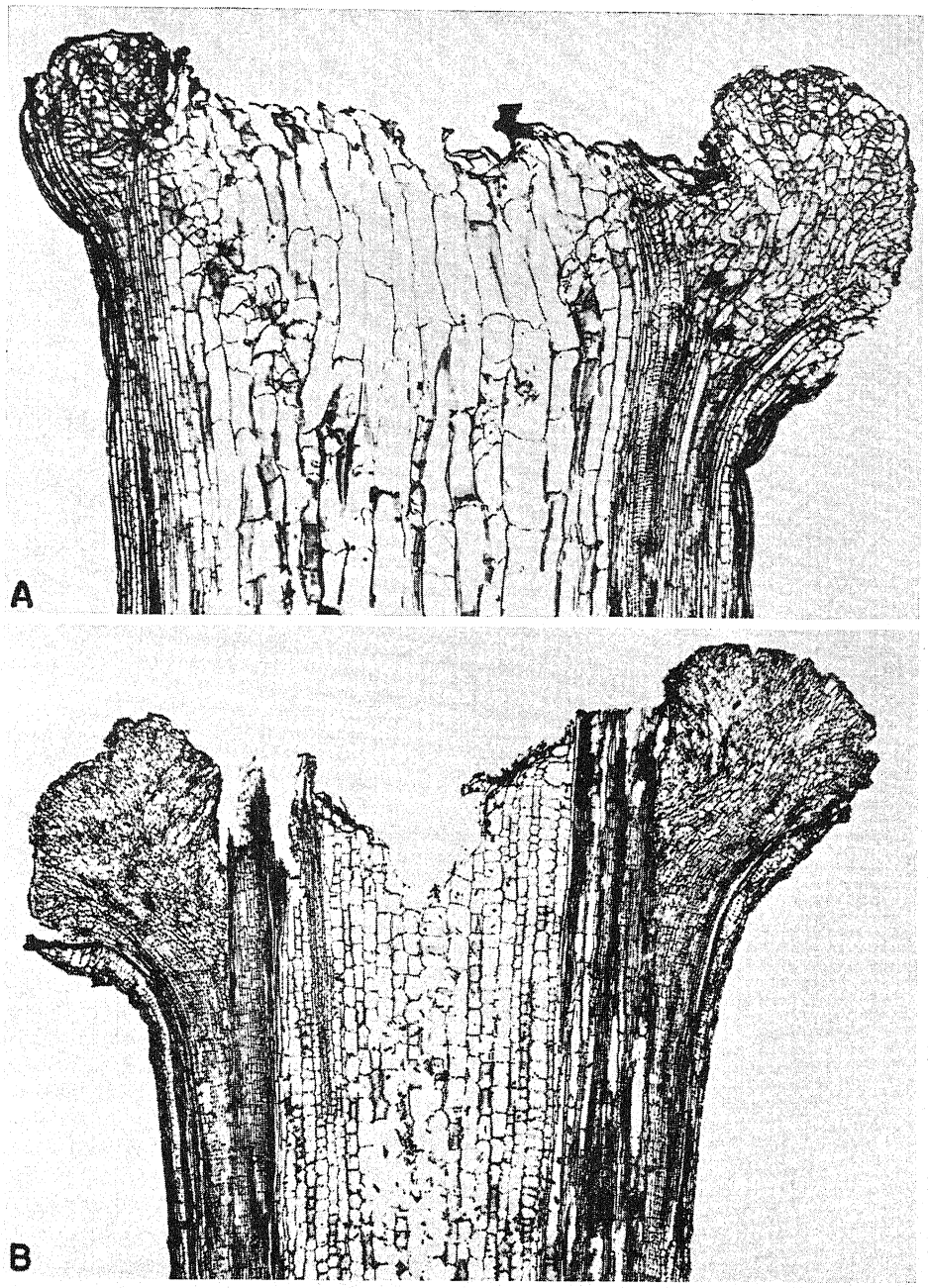


FIG. 12.—*A*, longisection of second internode from lot I, 7 days after decapitation, cut surface untreated. Superficial tumor derived mainly from activity in meristematic zone. Comparable in development to fig. 10. Traces of transverse phellogen. *B*, longisection of axillary branch from lot III, 9 days after decapitation, cut surface untreated. Proliferation of callus mainly in meristematic zone lying between well-developed secondary xylem and secondary phloem. Some proliferation in endodermis. As compared with *A*, greater proliferation of callus associated with greater activity of stem.

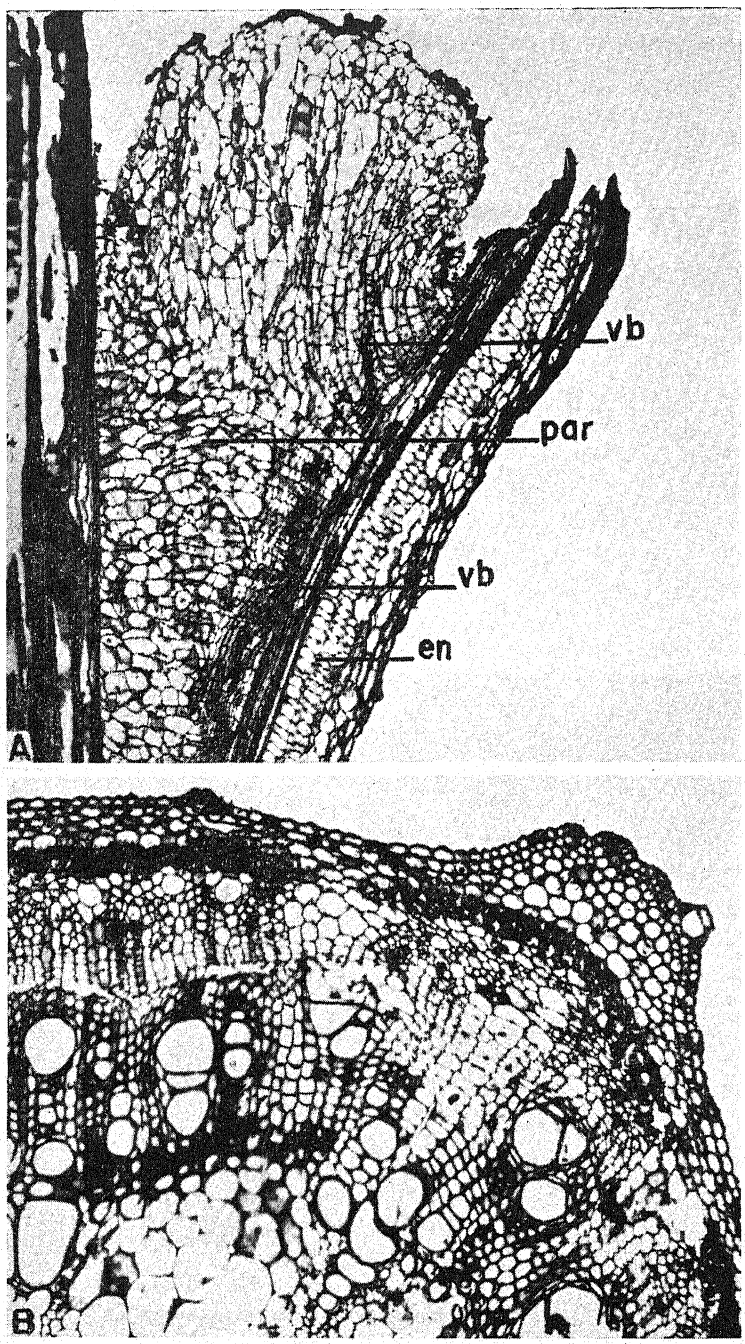


FIG. 13.—Axillary branches from lot III. *A*, longisection, 12 days after decapitation and treatment with lanolin only: *par*, parenchyma. Outer tissues inactive, except for slight proliferation in endodermis. Lignified pericyclic fibers showing no response. Proliferated, wedge-shaped meristematic zone lying between mature secondary phloem and secondary xylem and composed of vascular strands (*vb*) and parenchymatous tissue (*par*). *B*, detail of transverse section of axillary branch at time of decapitation. Mature stage of development shown in secondary tissues and heavily lignified pericyclic fibers. Compare with fig. 11*C*.

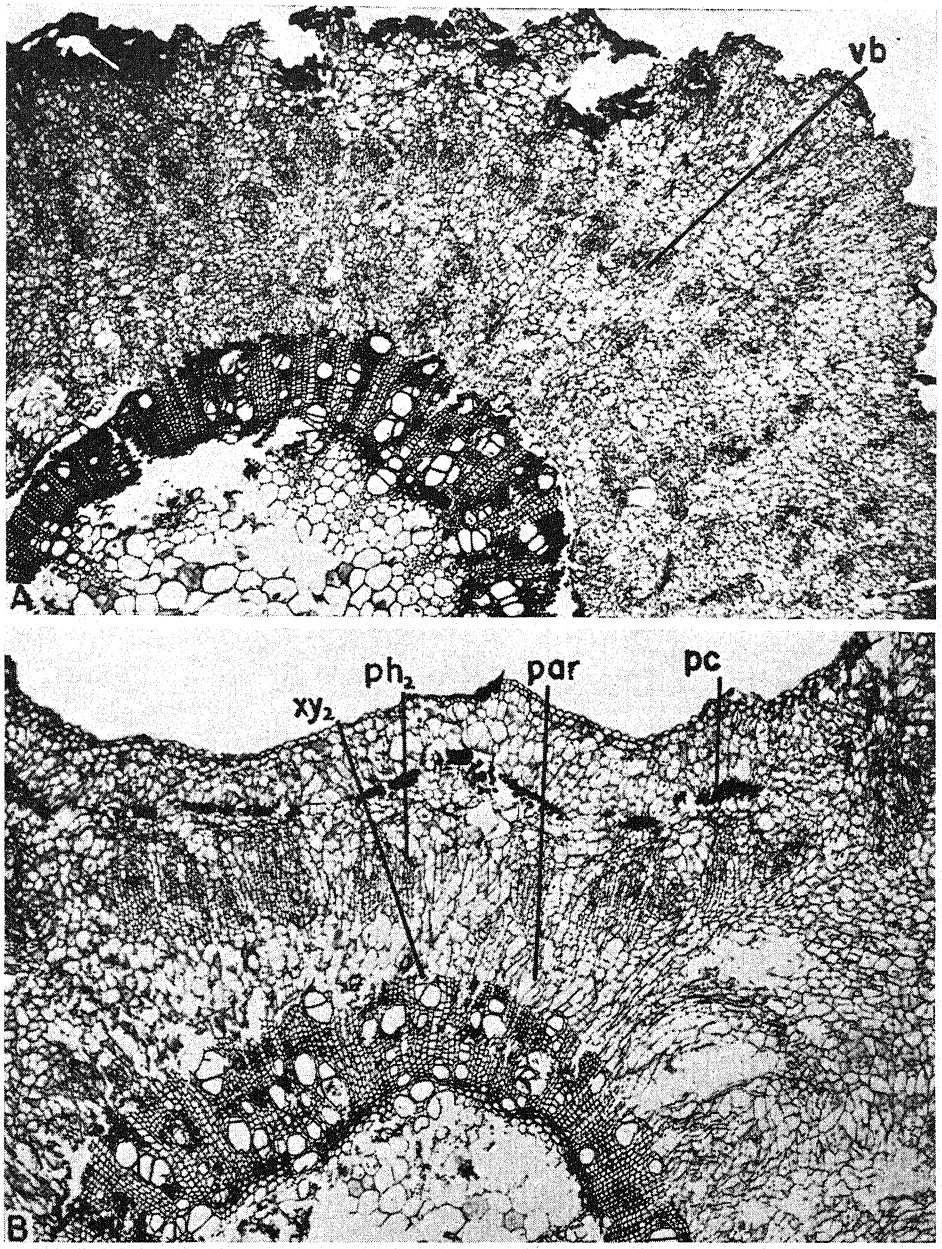


FIG. 14.—Transections of decapitated axillary branch from lot III, 9 days after treatment with lanolin. *A*, near cut surface through apical portion of callus. Outer tissues proliferated from phloem parenchyma and meristematic zone. Vascularization resulting in complex of bundles (*vb*). Inner cylinder consisting of secondary xylem formed before treatment and disintegrating pith. *B*, same stem, at level comparable to lowest level of fig. 13*A*. Little response in outer tissues; derivatives of meristematic zone (between ph_2 and xy_2) matured as vascular tissue or as parenchyma (*par*).

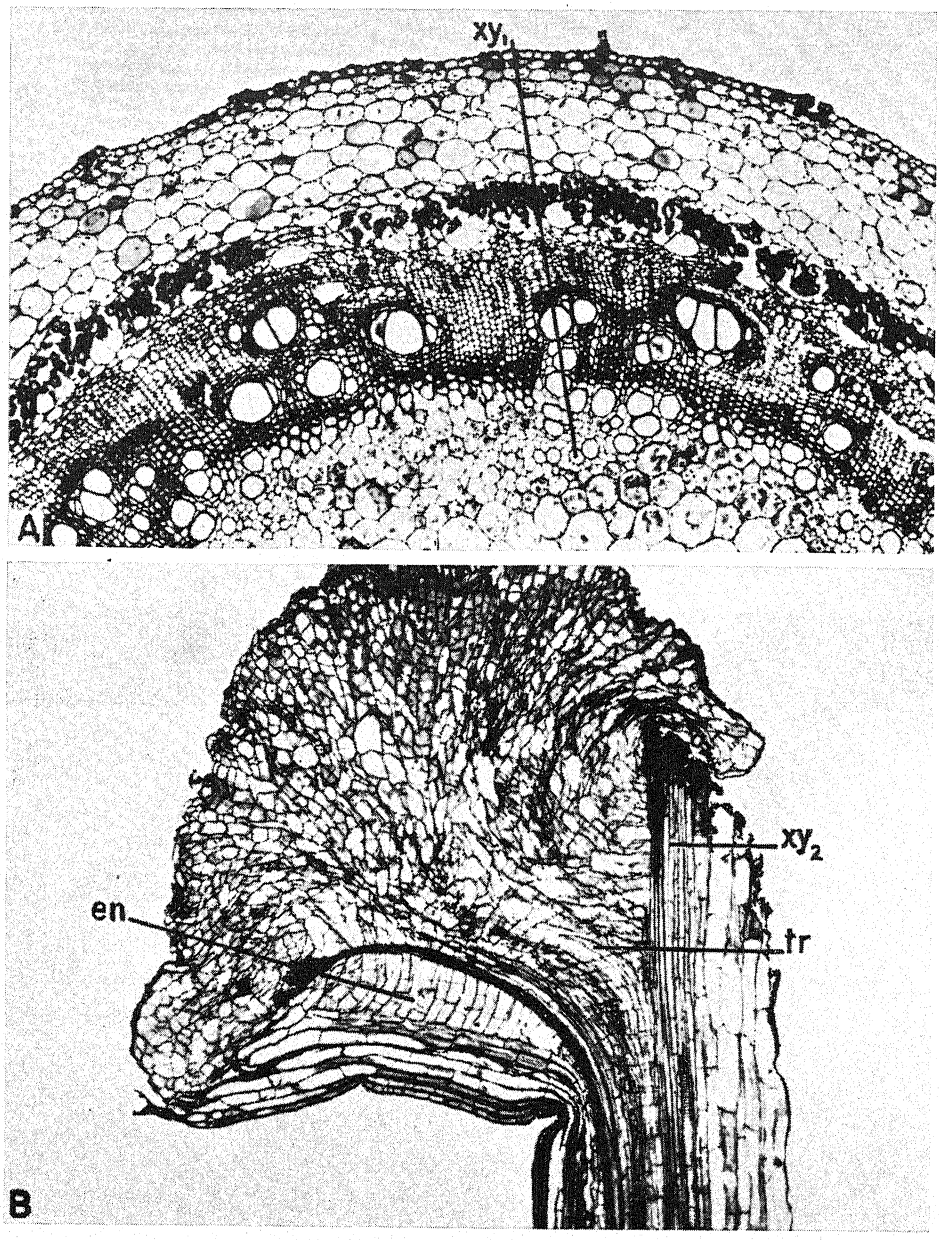


FIG. 15.—*A*, transection of hypocotyl below cotyledonary node. Mature stage of development, showing stemlike arrangement of tissues except for tangential arrangement of primary xylem (xy_1). *B*, longisection of hypocotyl from lot IV, 25 days after decapitation, cut surface untreated. Marked proliferation in endodermis; otherwise, development of callus similar to that in other control material. Note similarity to fig. 5*A* of material treated with nicotine.

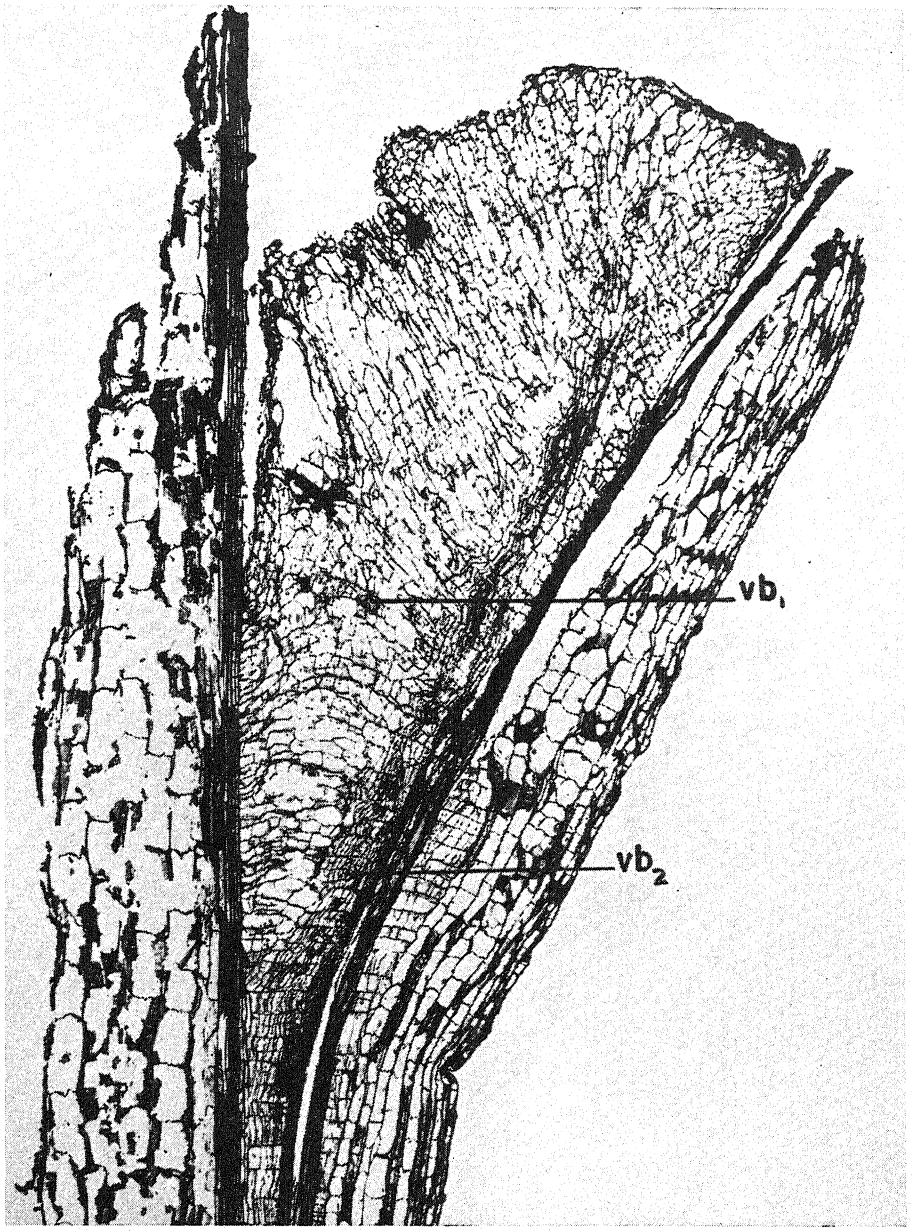


FIG. 16.—Longisection of hypocotyl, 15 days after decapitation, cut surface untreated. Wedge of proliferated tissue derived from meristematic zone lying between phloem and xylem formed in axis before treatment. Broad, upper portion proliferating to form outward flaring callus. Derivatives in proliferated area matured as: parenchyma; small vascular bundles (vb_1) scattered among parenchymatous cells; a well-defined vascular bundle (vb_2) formed along outer margin of callus and continuous with vascular tissues of axis.

tip, the orderly pattern of response is most clearly seen in longitudinal sections (figs. 2, 5*A*). Just below the cut surface was a zone of cells dividing in irregular planes. At lower levels divisions were limited to the tangential plane, and the derivatives appeared in successive rows, two to six cells deep. At still lower levels of response (between 2–3 mm.) was a region of simple radial enlargement. The endodermis did not form a highly meristematic layer and only infrequently initiated vascular tissue (fig. 4*B*). Generally, the derivatives matured as parenchyma. Little or none of the tumor was derived from the endodermis, although in some plants a few dividing cells at the cut surface might be associated with the proliferation of the much more active inner tissues (fig. 9*B*).

The response of pericycle to treatment with nicotine was influenced by the thickness of the cell walls at the time of decapitation. If the uppermost pericyclic cells were immature at this time, they might enlarge and become indistinguishable from adjoining tissues or they might be crushed beyond recognition (fig. 2). If these cells were partially matured, they were frequently crushed and by the 5th day appeared as a conspicuous dark line in both longitudinal (fig. 2) and cross sections (figs. 4*A*, *B*, 7*A*, *B*). In the latter, this crushed pericycle was clearly evident between 0.5 and 1.5 mm., especially over the bundles. At lower levels some of the pericyclic cells persisted uncrushed, and their walls thickened slightly. At increasing distance from the cut surface there was the usual maturation of fibers. The pericycle appeared to take no part in the formation of the tumor. Moreover, when the pericycle was crushed in the upper levels, outer tissues might be cut off from participation in tumor formation (fig. 5*A*).

Response in the upper levels of primary phloem parenchyma consisted of cell enlargement and divisions in various planes. This proliferation was like that of the cortical parenchyma, but it was greater in amount and contributed to the outward flaring of the stem tip. Enlargement and proliferation continued through the 5th day, the response finally extending to about 1.5 mm. below the cut surface (fig. 2). The derivatives matured as parenchyma. At the cut surface continued proliferation produced a small part of the peripheral portion of the tumor (fig. 9*A*, *B*).

Cells of the secondary phloem parenchyma, especially those in the phloem rays, responded in a manner similar to the primary phloem parenchyma. Enlargement and proliferation of the cells occurred in the upper levels and were completed by the 5th day. Sieve tubes and companion cells showed little activity and could be readily identified to within a few cells of the cut surface (fig. 2), although these sieve-tube groups might be disarranged by the activity of intervening phloem rays. As a secondary consequence of the proliferation of tissues centrad to the secondary phloem, sieve tubes and companion cells matured previous to decapitation might be crushed. This crushing became noticeable about the 9th day and, by the 12th day, resulted in patches of collapsed cells (fig. 7*B*). These were distinct at 1 mm. and extended to 2.5 mm. below the cut surface. Derivatives of secondary phloem became indistinguishable from those of the primary phloem in forming peripheral portions of the tumor above the cut surface.

The greatest degree of proliferation in response to treatment with nicotine was in the zone between mature secondary phloem and mature secondary xylem,

a region consisting of the cambium and the undifferentiated cells lying on either side of the cambium. Proliferation of these tissues resulted in an area of active tissue which is designated the meristematic zone. Cells of those portions of the interfascicular rays abutting the cambium and its derivatives likewise proliferated so that this zone was continuous about the stem (figs. 3*A*, 4*A*).

Proliferation in the meristematic zone was quickly initiated after treatment. By the 3d day cells in the uppermost levels had undergone divisions in various planes and had formed an irregular mass of tissue (fig. 1*A*). Between 0.5 to 1.5 mm. below the original level of the cut surface the undifferentiated derivatives were enlarged, often in a radial direction, and there was an increased number of cambial derivatives. By the 5th day a broad region of highly meristematic, generally proliferating, tissues had been formed at the stem tip (fig. 2). At lower levels this became a wide zone of radially arranged, apparently multinucleate cells. During the period of greatest proliferation successive divisions appeared to be so rapid that cell wall formation did not keep pace with nuclear division. When wall formation was completed later, the multicellular nature of the zone was clearly evident. With increasing distance from the cut surface the number of cell divisions and the width of the meristematic zone diminished. This zone was conspicuously wide at 1 mm. (fig. 4*A*) and much narrower (fig. 4*B*) but still evident at 2 mm. Activity in the meristematic zone resulted in the formation of a wedge-shaped ring of tissue (fig. 5*A*, *B*) which contributed largely to the outward flaring of the stem tip.

Below the original level of the cut surface maturation of cells in the meristematic zone began in 6 or 7 days and was first evident in the formation of relatively small, narrow, slightly elongated, reticulately thickened tracheids. Differentiation, as observed in longisections, was initiated along the curve of the outward flaring of the stem tip (fig. 5*A*). Outside this initial layer of tracheids the cells remained meristematic and formed an active and readily identified cambium. Cambial divisions resulted in additional tracheids so that by the 16th day a well-defined layer of tracheids was formed, often ten cells deep (figs. 5*B*, 9*A*, *B*). Outside the cambium a narrow layer of new phloem was differentiated (fig. 7*A*, *B*). At the lowest levels of response this layer of tracheids was adjacent to the xylem formed before treatment, and the newly formed xylem and phloem tissues merged with the new secondary tissues of unaffected portions of the stem. At the upper levels the proliferated tissues centrad to the tracheid layer generally matured as parenchyma. In the apical portions of the meristematic zone which proliferated above the original level of the cut surface, those cells near the periphery remained meristematic and proliferated actively to form the major part of the tumor.

Response of the xylem was dependent upon the formation and maturity of the cells at the time of decapitation. The degree of maturity varied from stem to stem and within a single stem. The first layer of secondary xylem differentiated in the bean stem consisted of fibers (4). In some of the treated stems this layer of fibers was matured almost continuously around the stem; in others it was interrupted across the rays or lacking over wide sectors (fig. 6*A*, *B*). Mature fibers and lignified vessels formed the ring or crown of xylem at the cut surface (fig. 2). These cells and elements showed little change following decapitation, except

that the very dark color resulting from the staining process suggested some change in chemical constitution. Because of differential activity, the tissues of the meristematic zone were frequently torn from this xylem layer. In the early stages of response this split might extend as far as 0.5 mm. below the cut surface (figs. 2, 3*A*). If the vessel segments, tracheids, and fibers were immature at the time of treatment, they might participate in the general apical proliferation. Somewhat below the cut surface (about 0.5 mm.) these immature elements often became enlarged; their cell walls were greatly stretched and in places might even be ruptured (figs. 3*B*, 4*A*). Cells of the xylem parenchyma adjacent to the vessels often enlarged, and in transection they formed a striking pattern encircling the vessels (fig. 1*B*, *C*). Frequently, the nucleus lay at the end of the parenchyma cell nearest the vessel. Response in the xylem parenchyma was evident by the 3d day. It might extend downward to 2 mm.

The interfascicular ray may be divided at the layer of fibers into an outer and an inner portion, the outer portion responding as part of the meristematic zone. At the cut surface the cells proliferated, and their derivatives became indistinguishable from those of the adjoining tissues (fig. 3*A*). At slightly lower levels, however, cells in this outer portion of the ray enlarged, especially in a radial direction. They might undergo a few tangential divisions, but no interfascicular cambium was formed. The derivatives matured as parenchyma. The large size of these cells distinguished the rays from other tissues proliferated in the meristematic zone (fig. 4*A*).

Response in the inner portion of the ray varied with the presence or absence of a layer of fiber. In general, when the fiber layer was present, only a few ray cells enlarged and underwent occasional divisions (figs. 3*B*, 4*A*). If the fiber layer was absent, the ray cells responded more generally with enlargement and division, especially those cells in close proximity to the meristematic zone (fig. 6*A*). The response in the inner portion of the ray appeared by the 3d day, and little change took place after the 5th day. The downward extent of the response varied; generally it was about 1 mm. At the cut surface the cells were frequently injured in treatment and did not participate in the formation of the tumor.

The cells of the pith showed no tendency to proliferate but began to collapse near the cut surface by the 3d day. This effect extended downward, and by the 5th day disintegration commenced in the center of the stem (fig. 2). Collapse of the pith resulted in a central depression characteristic of the gross aspects of the tumor. In older stages collapse of the pith might reach a depth of 1.5 mm. The central depression frequently became partially filled with tissue proliferated from the meristematic zone (fig. 9*A*).

The tumor was derived from the following tissues: a slight amount from the endodermis; a small part, mainly in the lateral portion, from primary and secondary phloem parenchyma; and a major portion from the highly responsive meristematic zone. That portion of the tumor which was above the cut surface resulted from the proliferation of these tissues. Upward growth was not distinct until the 5th day (fig. 2) and was pronounced by the 7th day (fig. 5*A*). The tumor increased in size for several weeks. The peripheral portions were small-celled and meristematic. In inner portions the tissue was parenchymatous (fig. 9*A*). Some cells might become very large and crush adjacent cells. The large cells might un-

dergo division, but often the walls were fragile and collapsed (fig. 6*B*). Vascularization was evident by the 12th day and was mainly a differentiation in apposition to the numerous vascular strands from the stem below. A complex system of branching vascular strands resulted. These were composed of short reticulate tracheids, narrow cambial-like cells, and small phloem-like elements. As maturation of the tumor proceeded, vascularization occurred in the peripheral portion, marginal cells lost their meristematic character, and exterior cells became suberized (figs. 5*B*, 8*A*, 9*A*, *B*).

No root primordia were observed in the material studied. The endodermis, the rays, and adjacent tissues did not reach a degree of proliferation and organization suggestive of the development of root histogens.

Histological examination of the stem tips treated in lot II confirmed early observation of gross material. The tumors formed as a result of treatment with 2% nicotine were small, while those formed after treatment with 3% nicotine were larger but not so vigorous as tumors in lot I. Both concentrations of nicotine produced tumors histologically similar to those already described in detail for lot I. However, associated with the weaker response of lot II was a lesser maturity of the stem tissues. This difference somewhat modified the appearance of proliferation at the stem tip. Especially significant was the absence of strongly developed xylem. The meristematic zone was not so clearly set off, and the ring or crown of protruding xylem elements was lacking at the cut surface.

CONTROL.—In the plants considered as controls the character of the response was consistent, despite differences in age and portion of plant involved (figs. 11*C*, 13*B*, 15*A*) and variations in time and extent of response. The limited proliferation observed in the stem tips of lots I and II (figs. 10, 11*A*, *B*, 12*A*) represented the initial stages of callus formation. In lots III and IV (figs. 12*B*, 13*A*, 15*B*, 16) proliferation advanced beyond these initial stages to the formation of a well-developed callus or tumor. The presence of lanolin on the cut surface tended to induce a greater degree of response than that which occurred in simply decapitated stem tips of comparable material. Because wound response in the control material was markedly similar to response following treatment with nicotine, a brief description of the former will suffice.

In the outer tissues of the stem the response was slight. The epidermis was injured, and the uppermost cells might undergo transverse divisions or otherwise show adjustment to internal expansion. The uppermost cells of the cortical parenchyma might be injured or might proliferate slightly (fig. 16). The endodermis varied considerably in the degree of response. It might show almost no change, particularly in cases of weak response of the stem tip (figs. 10, 11*A*, *B*, 12*A*). In other plants there might be characteristic radial elongation and tangential divisions of the endodermal cells. A layer several cells deep might result from these divisions (figs. 13*A*, 15*B*, 16). With very vigorous response a highly meristematic tissue was formed. The pericycle followed the pattern described for the nicotine treatment: at upper levels it might proliferate slightly (fig. 10) or be crushed (fig. 12*B*); at lower levels it matured as the usual fibers (fig. 14*B*).

The vascular and ray tissues responded in much the same manner as in plants treated with nicotine. At upper levels the parenchymatous cells of the primary and secondary phloem enlarged and prolifer-

ated to form the outer portions of the tumor or callus (fig. 14*A*). Matured sieve tubes and companion cells changed little and were readily identified in the enlarging stem tip. Again the activity of greatest significance in the formation of the wound callus or tumor was that of the cambium and adjacent undifferentiated tissues. The resulting meristematic zone was most active at the upper levels of the stem tip and gradually narrowed at lower levels to blend indistinguishably with normal secondary growth (fig. 16). As seen in longisection, this meristematic zone appeared as a wedge of proliferated tissue, with its narrow point lying between mature secondary phloem and secondary xylem and its broad portion forming the outward flaring callus or tumor (fig. 16). Maturation of this proliferated tissue occurred as in nicotine treatment. At levels below the original cut surface a cambium could be identified toward the outer or centrifugal side of the wedge. Activity of this cambium resulted in the formation of new phloem on the outside and new xylem tracheids on the inside. These tracheids were characteristically short, reticulately lignified, and often radially arranged. The remaining cells of the meristematic zone matured as parenchyma or differentiated scattered vascular bundles. The periphery of the tumor above the original level of the cut surface (fig. 15*B*), however, tended to remain meristematic and to continue proliferation with proportionate increase in the size of the tumor.

The pith and the xylem formed previous to decapitation showed little activity. Lignified xylem elements at the stem tip protruded as the ring or crown visible in gross observations (figs. 13*A*, 16). Immature or parenchymatous cells of the xylem, especially in younger stems, might enlarge and proliferate slightly near the cut surface (fig. 10). The cells of the pith generally collapsed following decapitation, although in younger stems a transverse phellogen might form (figs. 10, 11*C*, 12*A*).

Discussion

In the present experiment the response of control bean plants to decapitation conformed to the description of wound response and callus formation summarized by KÜSTER (11) for plants in general. A dominant role was assigned to the cambium in the formation of a wedge-like ring of callus tissue for which KÜSTER used the term "Lohden-wedge" as originally given by HARTIG. KÜSTER stated that the activity of the pith and phloem was variable, although in some plants the latter was scarcely less active than the cambium. He likewise noted the inequality of response over different parts of the cut surface. SCHILBERSZKY (19), in more detailed studies of wound response in species of *Phaseolus*, also described the formation of a strong callus from the region of the cambium. Under special conditions of wounding, he recorded the formation of a meristematic layer derived from the endodermis and producing well-developed extrafascicular bundles. This degree of development was not attained in the specimens of the present study.

In both the plants treated with nicotine and those considered as controls, the degree of response differed considerably from lot to lot. These differences are ascribed to factors in the environment or to the nutritive status of the plant. Both SCHILBERSZKY and KÜSTER have noted the significance of such factors as moisture and food supply in causing variation in the degree of natural wound response. FLINT and MORELAND (6) reported striking differences in natural wound tumors

under varying conditions of humidity and light. The experiments of BAUSOR (1), BEAL (2), and MITCHELL and BROWN (14) have shown the interrelationship between the reaction to growth-regulating substances and the nutrition of the plant. In the present experiment environmental factors, such as light, were more favorable for the nutrition and growth of plants in lot I than for those in lot II. The significance of age was apparent among control plants, in which response was greater in the axillary branch and hypocotyl than in the young stem tip. On the basis of histological observations of all the lots, greater response was associated with vigorous growth and a greater maturity of stems.

It is difficult to evaluate the position of nicotine as a growth-regulating substance. Nicotine in the form of nicotine sulphate has been repeatedly applied in great dilution as an insecticide with no apparent effect on the plant. Experiments in which nicotine has been supplied to the plant in nutrient solution have produced results which have ranged from toxicity to stimulation. As a result of experiments with rooting, TRAUB (21) placed nicotine in the same category as the highly stimulative indolebutyric acid. Yet in the series of histological studies on bean, nicotine differs markedly from the response described by HAMNER and KRAUS (7) for indolebutyric acid, both in the character of the tumor and in the absence of root primordia. Root formation also separates response with nicotine from response with indoleacetic acid, naphthaleneacetic acid, α-naphthalene acetamide, and some substituted phenoxy compounds. Response with nicotine was more like that with tryptophane and tetrahydrofurfuryl butyrate, in which root primordia were absent, and with phenylacetic acid, in which root formation was infrequent. However, root formation cannot be considered an absolute criterion in evaluating responses. Not only has TRAUB recorded root formation with nicotine—under different circumstances and with different plant material—but also recent observations with various concentrations of indoleacetic acid (16) indicate that the presence or absence of root primordia may be dependent upon the concentration of growth-regulating substance used.

The pattern of response induced by various growth-regulating substances, the degree of proliferation, and the type of maturation in the tissues separate response to one growth-regulating substance from response to another substance. Particularly significant are the tissues showing the greatest degree of activity. With nicotine this was the area of the cambium, designated the meristematic zone. The pith was highly responsive to indoleacetic and indolebutyric acids and less so to naphthaleneacetic acid. All three resulted in conspicuous activity in the endodermis, the phloem, and the rays. Application of α-naphthalene acetamide caused a marked increase in strongly lignified secondary xylem. With phenylacetic acid and with tryptophane, activity appeared to be greatest in the phloem and endodermis with the development of well-defined vascular bundles, especially with tryptophane. Tetrahydrofurfuryl butyrate induced a response similar in some ways to that resulting from nicotine. With tetrahydrofurfuryl butyrate the cambium was greatly activated in the production of xylem, but there was proliferation of xylem parenchyma and pith at later stages.

The response induced by nicotine in the present experiment most closely resembled that resulting from wounding. In both responses the greatest prolifera-

tion occurred in the meristematic zone originating from the cambium and adjacent undifferentiated tissues. Tumor formation and vascularization were also essentially alike. However, there were differences in the two responses. Nicotine not only considerably augmented the amount of response beyond that which occurred naturally under comparable circumstances but it also induced a response under conditions where the natural response failed to occur. With nicotine, tumor formation proceeded at a predictable rate. With natural wound response a tumor might be formed in 7 days, 21 days, or not at all. These properties of nicotine may be considered significant: nicotine induces a response histologically similar to wound response, and it has the capacity to induce response in greater degree and with greater certainty than in comparable cases of natural wounding.

Summary

1. Nicotine, applied in lanolin to the decapitated second internodes of young Red Kidney bean plants, induced a well-defined response with the formation of a superficial tumor at the stem tip.

2. The tumor was flat-topped with peripheral portions growing downward and closely encircling the stem. The surface was finely tuberculate. There was no evidence of root primordia.

3. Histological characteristics were as follows: (*a*) slight proliferation of cortical parenchyma at cut surface; (*b*) enlargement of endodermal cells with several tangential divisions near the cut surface; (*c*) pericycle crushed or maturing as fibers; (*d*) general proliferation of phloem parenchyma at cut surface; sieve tubes little changed; (*e*) greatest activity in cambium and adjacent undifferentiated cells forming a meristematic zone and resulting in a wedge-shaped area of proliferated tissue; at the apex this tissue continued to proliferate to form a major portion of the tumor; derivatives matured as parenchyma or as a complex of vascular bundles, the largest of which lay along the outer margin of the wedge and were continuous with the vascular tissues of the stem axis; (*f*) in the xylem no activity in the lignified elements; some enlargement of the parenchyma; (*g*) outer portion of rays proliferated as part of the meristematic zone; mainly cell enlargement in inner portions; (*h*) pith inactive and soon disintegrating; (*i*) tumor above the treated surface derived mainly from the derivatives of the meristematic zone; small, peripheral portions from proliferation of the phloem.

4. Observations of wound response were made on decapitated axes of various parts of bean plants. Young decapitated stems responded with weak, irregular tumors or failed to form any callus. Hypocotyls and decapitated older branch axes responded with well-defined callus formation.

5. The histological character of wound response in the bean conformed to that reported for plants in general.

6. The response to nicotine showed greater similarity to wound response than to responses described for various growth-regulating substances.

LITERATURE CITED

1. BAUSOR, S. C. Interrelation of organic materials in the growth substance response. BOT. GAZ. 103:710–724. 1942.
2. BEAL, J. M. Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. 102:366–372. 1940.
3. ———. Reactions of decapitated bean plants to certain of the substituted phenoxy compounds. BOT. GAZ. 108:166–186. 1946.
4. DOUTT, MARGARET T. Anatomy of *Phaseolus vulgaris* L. var. Black Valentine. Michigan State College Agr. Exp. Sta. Tech. Bull. 128. 1932.

5. EISLER, M., and PORTHEIM, L. Weitere Untersuchungen über die Nikotinvergiftung von Früchten und Samen. Planta. 8:1–26. 1929.
6. FLINT, LEWIS H., and MORELAND, CHARLES F. Note on gall formation in decapitated young bean plants. Plant Physiol. 20:453–456. 1945.
7. HAMNER, K. C., and KRAUS, E. J. Histological reactions of bean plants to growth promoting substances. BOT. GAZ. 98:735–807. 1937.
8. KRAUS, E. J. Histological reactions of bean plants to l-tryptophane. BOT. GAZ. 102:602–622. 1941.
9. KRAUS, E. J.; BROWN, NELLIE A.; and HAMNER, K. C. Histological reactions of bean plants to indoleacetic acid. BOT. GAZ 98:370–420. 1936.
10. KRAUS, E. J., and MITCHELL, J. W. Histological and physiological responses of bean plants to alpha naphthalene acetamide. BOT. GAZ. 101: 204–225. 1939.
11. KÜSTER, ERNST. Pathologische Pflanzenanatomie. Jena. 1925.
12. MACHT, DAVID I., and DAVIS, MARY E. Toxicity of alpha- and beta-nicotines and nornicotines for *Lupinus albus*. Amer. Jour. Bot. 22:329–332. 1935.
13. MARTIN, HUBERT. The scientific principles of plant protection. London. 1940.
14. MITCHELL, JOHN W., and BROWN, JAMES W. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. BOT. GAZ. 107:393–407. 1946.
15. MULLISON, WENDELL R. Histological responses of bean plants to tetrahydrofurfuryl butyrate. BOT. GAZ. 102:373–381. 1940.
16. MURRAY, MARY AILEEN, and WHITING, A. GERALDINE. A comparison of histological responses of bean plants to tryptophane and to low concentrations of indoleacetic acid. BOT. GAZ. 108 74–100. 1946.
17. NEMEC, B. Über den Einfluss des Nikotins auf sich teilende Zellen. Protoplasma 7:99–105. 1929.
18. RICHARDSON, H. H.; BULGER, J. W.; BUSBEY, R. L.; NELSON, R. H.; and WEIGEL, C. A. Studies on nicotine fumigation in greenhouses. U.S.D.A. Circular 684. 1943.
19. SCHILBERSZKY, KARL. Künstlich hervorgerufene Bildung secundäre (extrafasciculärer) Gefässbündel bei Dicotlyedonen. Ber. Deutsch. Bot. Ges. 10:424–432. 1892.
20. SONNENSCHEIN, CL. New experiments on the breeding of polyploid forms of soybeans. Forschungsdienst. 12:532– 37. 1941. (Chem. Abst. 1943. Col. 3799.)
21. TRAUB, HAMILTON P. Growth substances with particular reference to subtropical fruit plants. Proc. Amer. Soc. Hort. Sci. 35:438–442. 1938.
22. WEIS, F.; BLOM, J.; and NIELSEN, N. The ability of corn plants to absorb and endure different poisonous compounds. International Corn Borer Investigations Scientific Reports 1927–28. Pp. 153–173. International Livestock Exposition. 1928.
23. WHITING, A. GERALDINE, and MURRAY, MARY AILEEN. Histological responses of bean plants to phenylacetic acid. BOT. GAZ. 107:312–332. 1946.
24. WITHROW, ALICE P., and BIEBEL, J. P. Nicotine fumigation injury in Biloxi soybean. Phytopath. 34:256–257. 1944.

EFFECTS OF PRE-STORAGE TREATMENT OF HARDWOOD AND PINE SEEDLINGS WITH α-NAPHTHALENEACETIC ACID

ROGER D. WAY AND T. E. MAKI[1]

Introduction

Although forest tree seedlings usually are planted within a few days after lifting from the nursery bed, storage of stock frequently becomes necessary when, for instance, fall lifting for early spring planting is desirable, or when planting of lifted stock is delayed by such circumstances as a prolonged period of bad weather or a shortage of labor. The principal objective of storage is to maintain dormancy or to hold growth to a minimum without injury to, or deterioration of, the stock. Where refrigerated storage is available, this problem is seldom acute, but where common storage facilities, such as an unrefrigerated cellar or shed, or outdoor heeling-in, must be relied upon, the length of time over which stock can be held without deterioration is definitely limited.

[1] Formerly Research Assistant and Forester, respectively, Northeastern Forest Experiment Station, U.S. Forest Service, Philadelphia, Pa.

In current methods of seedling storage, emphasis has been placed primarily upon proper control of temperature and humidity in the storage chambers (1, 3, 7). Though those factors undoubtedly are of paramount importance, recent advances in the practical application of growth-regulators to various fields of plant culture have spurred hope that these chemicals might also prove helpful for maintaining or prolonging dormancy of seedlings in storage. Recent studies (4, 5, 6) have shown that shoot growth of seedlings, both in the field and in storage, can be inhibited by use of various growth-regulators. Accordingly, several experiments were undertaken at the Beltsville Forest Laboratory[2] to determine the practical utility of growth-regulator treatments for prolonging the time that tree seedlings can be held in common storage. The present paper summarizes the results from tests with naphthaleneacetic acid.

Review of literature

MARTH's (5) extensive work on roses is the first published study known to the writers that deals with growth-regulator treatments of woody plants in storage. He found that naphthalenemethylacetate, naphthaleneethylacetate, and naphthaleneacetonitrile, applied either as vapor or as spray in proper concentrations, inhibited top growth and conserved the starch reserve of the stock during storage. When outplanted, stock treated prior to storage showed increased root growth, delayed top growth, but, at the end of the season, greater total shoot growth than untreated plants. In the vapor state the most effective concentrations of chemical were 0.3–0.4 mg./cu. ft. of treatment chamber; optimum periods of exposure were about 16 hours at 32°, 4 hours at 40°, or 1 hour at 70° F. Concentrations of vapor above optimum caused injury; concentrations below optimum stimulated shoot development in storage. For use as a spray MARTH recommended a concentration of 0.01% in 0.25% wax emulsion. The most striking effects of the treatments were in preventing deterioration of stock in common storage; however, they also improved the field performance of stock kept in controlled cold storage.

In a series of exploratory experiments begun in the autumn of 1942 at the Beltsville Forest Laboratory, OSTROM (6) obtained highly promising results with hardwood stock held in unrefrigerated storage for late planting. Both vapors and sprays of naphthalenemethylacetate largely prevented development of etiolated shoots of white ash. Dormancy was prolonged for a month or more indoors but for only short periods in an outdoor heeling-in bed. Root initiation in these two types of storage was stimulated both above and below the root collar. Vapors and sprays on white ash held in unrefrigerated storage improved field survival. Treated stock, kept fully dormant in cold storage and planted late, showed a temporary delay in leafing-out and higher survival in the plantation than untreated stock.

The experiments discussed in this paper were in general a continuation and expansion of OSTROM's work.[3]

Material and methods

The present study was designed to determine the effects of method of applica-

[2] A branch of the Northeastern Forest Experiment Station, near Laurel, Md.

[3] These experiments were conducted in co-operation with the Hormone Project of the Bureau of Plant Industry, Soils and Agricultural Engineering. Special thanks are due Dr. CHARLES L. HAMNER, who prepared the chemicals and assisted in treating and storing the stock.

tion and of concentration of naphthaleneacetic acid as an agent for prolonging dormancy of hardwood and pine seedlings in common storage. It involved two separate schedules of pre-storage treatments and two field plantings. In the first experiment, tests were made of the effects of three methods of applying the growth-regulator (emulsion-dip, vapor, aerosol) on seedlings of black locust (*Robinia pseudoacacia* L.) and loblolly pine (*Pinus taeda* L.). The second experiment tested the effects of a range of concentrations of the growth-regulator, applied in emulsions only, on seedlings of red oak (*Quercus borealis* var. *maxima* Marsh.) and red pine (*P. resinosa* Ait.). Limited quantities of suitable stock necessitated using different species for the two phases of the study. Both plantings were in an infertile sandy loam soil of an abandoned field.

Treatment effects were determined on the basis of seedling response in terms of prolongation of dormancy, survival, vigor, and terminal growth.

Investigation

Experiment I. Method of application

The efficacy of emulsion-dip, vapor, and aerosol methods of growth-regulator applications were compared. For the emulsion-dip method, in which lanolin at 5 gm./l. of water was used as the growth-regulator carrier, preparation consisted first of dissolving naphthaleneacetic acid in melted lanolin in proportions to give concentrations of 100 and 500 mg. of the acid per liter of emulsion. Then the lanolin-acid mixtures were emulsified in the required amounts of water, using monoethanolamine stearate at 10 gm./l. as the emulsifying agent. A third emulsion, prepared as above except that no growth-regulator was added, served as a check of carrier effects. A fourth, with the same concentration of lanolin but emulsified with laundry soap at 10 gm./l., provided a comparison of the effects of emulsifying agents. In treating by the dip method, bundles of seedlings were immersed, tops down, into the liquid to the level of the root collars, were gently agitated to assure complete coverage of all twigs and foliage (of pines), and then were removed and allowed to drain a few minutes before packing for storage.

In the vapor method alcoholic solutions of naphthaleneacetic acid were vaporized on a hot plate in an airtight chamber containing the bundles of seedlings. The latter were exposed to the vapors for 16 hours at about 70° F. at concentrations of 0.4 and 0.6 mg./cu. ft. of chamber space. Control seedlings of the vapor series provided the only untreated group in the test.

Aerosol treatments were applied by the method used by Hamner, Schomer, and Goodhue (2). The aerosol mixture consisted of 1% naphthaleneacetic acid dissolved in 10% cyclohexanone (a mutual solvent for the hormone) and 89% dimethylether (b.p. = 27° C.), which is a liquefied gas and develops 89–90 lb./sq. in. pressure at room temperature and thereby disperses the hormone through a capillary jet, forming an aerosol. The aerosol was released into an airtight chamber containing the bundles of seedlings. As with the vapor treatments, concentrations of the growth-regulator were 0.4 and 0.6 mg./cu. ft. of space, and exposure was for 16 hours at about 70° F. Control seedlings of the aerosol series were exposed to a similar aerosol mixture containing no growth-regulator. For both vapor and aerosol treatments seedling roots were packed in damp sphag-

num wrapped in burlap to prevent drying during the treatment period.

The stock used in these tests was 1-year-old black locust and loblolly pine, lifted, respectively, on April 1 and April 10. For each species, 2000 seedlings were sorted into eighty uniform bundles of twenty-five seedlings each. This provided four replications for each treatment. The complete array of treatments consisted of three methods of application (emulsion-dip, vapor, and aerosol) × three levels of naphthaleneacetic acid (none, medium, and high) × two planting dates (May 12 and May 25), plus one soap-emulsion dip treatment for each planting date, making a total of twenty treatments per species.

Treatments by all three methods were applied on April 11, 12, and 13, 1944. The bundles of seedlings were packed immediately after treatment into bushel baskets with moist sphagnum around the roots and were placed in common storage. The storage house was a half-cellar of masonry walls below ground level and insulated frame construction above ground, with an adjustable ventilator in the roof. Temperatures in the house during storage gradually rose as follows: April 12, 51°; April 22, 53°; May 10, 60°; May 22, 65°; and May 23, 67° F.

The first planting was made on May 12 and 13 and the second on May 25 and 26. The plantation arrangement consisted of four randomized blocks, each split into two plots assigned at random to the two species. The twenty treatments were randomized row-wise within each plot. Rows were 3 feet apart, each containing twenty-five seedlings spaced 2 feet apart. Planting was done by the slit method, using a standard type of tree planting bar.

RESULTS

BLACK LOCUST.—Seedlings of the first planting, when removed from storage on May 10, were still almost completely dormant. Only a few buds had elongated as much as an inch. In contrast, seedlings removed from storage 13 days later (May 23), during which time temperatures in the house ranged between 60° and 67° F., had grown remarkably. Some shoots were 2.5 feet long. Of the several growth-regulator treatments, only the emulsion with 500 mg. of naphthaleneacetic acid per liter had inhibited growth sufficiently to be readily evident upon casual inspection.

The number of shoots over half an inch long was counted on each seedling in the first planting on May 15 and on each seedling in the second planting on May 29. The mean numbers per seedling were, respectively, 1.8 and 3.7; the mean difference of 1.9 is significant at the 1% level. Thus, the extra 13 days in storage had resulted in a marked increase in number as well as in length of shoots. Effects of the treatments on number and total length of shoots on seedlings of the second planting are shown in table 1. Treatment effects on the first planting were generally similar to those on the second planting. Seedling response to the soap emulsion was not different from that produced by the monoethanolamine stearate emulsion.

Inhibiting effects of naphthaleneacetic acid on shoot production were in proportion to its concentration except in the vapor method, where the medium level, for some obscure reason, inhibited shoot production more than the high level. The high-level emulsion was particularly effective. However, the lack of shoots may have been due to outright killing of leaf primordia rather than to true inhibition of shoots.

Survival of seedlings was recorded on July 6 and November 4, 1944, and on May 18, 1945. Since treatment effects were practically identical in the three tallies, only those shown in the last tally are discussed. Neither the method of application nor concentration of the acid caused any significant differences in survival. However, planting dates had a highly significant effect. Seedlings planted May 12 and May 25 showed average survival of 92.4% and 76.4%, respectively. The mean difference of 16.0% is significant at the 1% level.

TABLE 1

INHIBITING EFFECTS OF PRE-STORAGE TREATMENTS WITH NAPHTHALENEACETIC ACID ON SHOOT GROWTH OF BLACK LOCUST SEEDLINGS HELD IN COMMON STORAGE, APRIL 12–MAY 23. SEEDLINGS PLANTED MAY 25, EXAMINED 4 DAYS LATER. BASIS: 100 SEEDLINGS PER MEAN

Method of application	Concentration of acid	Mean no. of shoots over ½ inch long per seedling	Mean total shoot length per seedling (in.)
Lanolin emulsion..	0	4.2	29.8
	100 mg./l.	3.9	28.7*
	500 mg./l.	2.6†	23.1†
Vapor.....	0	4.0	34.5
	0.4 mg./cu. ft.	3.3*	27.5†
	0.6 mg./cu. ft.	3.8	28.4*
Aerosol.....	0	4.0	34.9
	0.4 mg./cu. ft.	3.7	25.0†
	0.6 mg./cu. ft.	3.7	21.1†

* Inhibiting effects significant at 5% level.
† Inhibiting effects significant at 1% level.

In general, seedling vigor and growth were closely correlated with survival. Vigor was rated according to an arbitrary scale as excellent, good, poor, and very poor. The average proportions of seedlings in excellent vigor on July 6, 1944, were 64.8% for the early and 19.6% for the late planting. The mean difference of 45.2% is significant at the 1% level. Neither methods of application nor levels of naphthaleneacetic acid had any significant effect on over-all seedling vigor. The treatments, however, did influence survival and vigor of the long, etiolated shoots which had developed on seedlings of the May 25 planting during the latter part of the storage period. Though most of these spindly shoots became green and persisted for some time after planting, many of them died later in the season (fig. 1). All growth-regulator treatments and even emulsion alone tended to increase both the percentages of spindly shoots remaining alive and, especially, the percentages displaying excellent vigor (table 2).

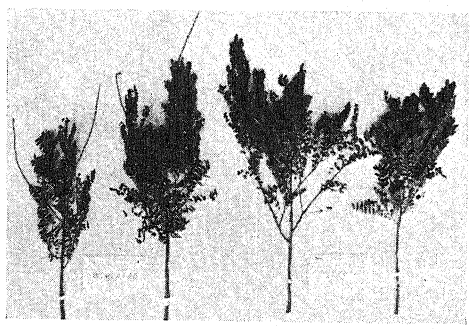


FIG. 1.—Effect of naphthaleneacetic acid in preventing development of spindly shoots on black locust seedlings held in common storage for 6 weeks, then planted on May 25, 1944: *left*, untreated seedlings; *right*, seedling tops dipped into 0.5% lanolin emulsion containing 100 mg. of acid per liter. Note dead spindly shoots on untreated seedlings. Photo taken July 26, 1945.

Both shoot elongation and foliage development were, like survival and over-all vigor, unaffected by the growth-regulator treatments but were strongly influenced by date of planting. At the end of the first growing season, total lengths of new growth were significantly greater for the early than for the late planting, despite the in-storage develop-

ment of etiolated shoots up to 2.5 feet long on the late-planted series. Thus, by the end of the first season, the early planting was definitely superior not only in quality but also in amount of new shoot growth. On May 18 of the second season (1945), mean seedling heights were 18.1 inches for the early and 14.5 inches for the later planting. On the same

TABLE 2

EFFECT OF PRE-STORAGE TREATMENTS WITH VARYING CONCENTRATIONS AND METHODS OF APPLICATION OF NAPHTHALENEACETIC ACID ON SHOOT GROWTH OF BLACK LOCUST SEEDLINGS HELD IN STORAGE. PLANTED MAY 25, EXAMINED JULY 6. BASIS: 100 SEEDLINGS PER MEAN

Method of application	Concentration of acid	Spindly shoots living (%)	Spindly shoots in excellent vigor (%)
Lanolin emulsion..	0	63.2	30.0*
	100 mg./l.	72.2	32.8*
	500 mg./l.	73.2†	25.2*
Vapor.....	0	58.0	12.5
	0.4 mg./cu. ft.	69.2	24.5*
	0.6 mg./cu. ft.	75.0†	25.0*
Aerosol.....	0	42.2‡	10.8
	0.4 mg./cu. ft.	68.2	30.5*
	0.6 mg./cu. ft.	78.2*	40.0*

* Benefits significant at 1% level.
† Benefits significant at 5% level.
‡ Harmful effects significant at 5% level.

ing storage. Mean terminal bud length in the May 12 planting was 0.83 inch and in the May 25 planting, 0.94 inch. The mean difference of 0.11 inch is significant at the 1% level. The growth-regulator treatments, except for the high-level vapor, did not retard bud elongation in storage in any consistent manner (table

TABLE 3

EFFECTS OF PRE-STORAGE TREATMENTS WITH NAPHTHALENEACETIC ACID ON MEAN LENGTH AND VIGOR OF TERMINAL SHOOTS OF LOBLOLLY PINE SEEDLINGS. BASIS: 200 SEEDLINGS PER MEAN (BOTH PLANTING DATES COMBINED)

Method of application	Concentration of acid	Mean terminal bud length (in.)	Seedlings with vigorous terminal buds (%)
Lanolin emulsion..	0	0.91	66.5
	100 mg./l.	.90	41.0*
	500 mg./l.	.89	3.0*
Vapor.....	0	.93	63.0
	0.4 mg./cu. ft.	.88	79.0†
	0.6 mg./cu. ft.	.79‡	78.0†
Aerosol.....	0	.81‡	80.5§
	0.4 mg./cu. ft.	.93	70.0
	0.6 mg./cu. ft.	0.92	64.0

* Harmful effects significant at 1% level.
† Vigor benefits significant at 5% level.
‡ Inhibiting effects significant at 5% level.
§ Vigor benefits significant at 1% level.

date, 79.2% of the early planted seedlings showed good foliage development (ten or more well-developed leaves per plant) as against 55.6% for the later planting. These differences in height and foliation are statistically significant at the 1% level.

LOBLOLLY PINE.—Measurements were made of the lengths of terminal buds on all loblolly pine seedlings 3 and 4 days after the first and second plantings, respectively. Planting dates had a highly significant effect on bud elongation dur-

3). The apparent retardation in the aerosol-0 lot may represent chance variation or possibly indicates inhibitory effects of the carrier which were neutralized or counterbalanced in the other two lots by possible stimulating action of the growth-regulator.

The number of seedlings showing vigorous growth of terminal buds a few days after planting was not affected by planting date. The chemical treatments, however, exerted some marked effects on bud vigor (table 3), the acid in emul-

sions being injurious, the other treatments being either neutral or in some degree beneficial.

Survival of loblolly pine seedlings was recorded on May 16, June 29, and November 6, 1944, and on June 19, 1945. Although survival was less for each succeeding tally, treatment relationships underwent no substantial changes; hence, results of only the last observation are discussed.

During the month of May when the plantings were made, the rainfall was nearly 2 inches below, and the mean temperature was 6.0° F. above, the 35-year average. These abnormally dry-and-hot weather conditions resulted in severe mortality of loblolly pine. Winter mortality, though some occurred, was not unduly high.

As with locust, planting dates had a highly significant effect on survival of loblolly pine. Average survival of seedlings planted May 12 was 32.8%; for those planted May 25, 22%. The mean difference of 10.8% is significant at the 1% level.

Results of growth-regulator treatments upon survival are shown graphically in figure 2. The most striking feature is the highly injurious effect of the chemical when applied in emulsions. Results with all other treatments were suggestive of beneficial or injurious tendencies, but only the 0.4-mg. concentration in vapor showed enough difference from controls to be recognized statistically; that difference barely attained significance at the 5% level. A reasonably close parallel is evident between the figures on bud vigor (table 3) and on survival (fig. 2).

By mid-May of the second season, mean height of seedlings in the early planting was significantly greater (at the 1% level) than in the late planting (10.8 *vs.* 9.0 in.). Accurate evaluation of other treatment effects on height was rendered difficult because of the heavy losses and resultant unequal weightings of means. The growth-regulators in emulsions unquestionably had retarded growth; for all other treatments no appreciable differences in growth were apparent.

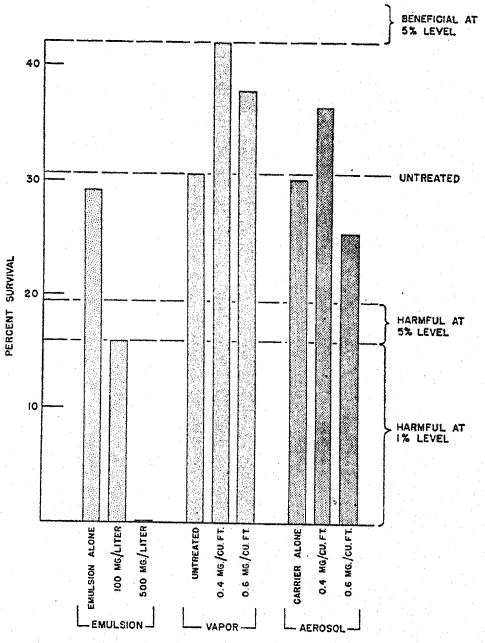


FIG. 2.—Effect of method of application and of concentration of naphthaleneacetic acid on survival of 1-year-old loblolly pine seedlings (planting dates combined). Zero level in vapor series is only untreated group since zero level in other two series represents carriers, aerosol (cyclohexanone and dimethylether) and lanolin emulsion, respectively.

EXPERIMENT II. CONCENTRATION OF CHEMICAL

The primary purpose of this phase of the study was to gain more precise information on the efficacy of different concentrations of naphthaleneacetic acid than could be drawn from the tests in experiment I. Methods of application

were restricted to dipping in lanolin emulsions carrying varying amounts of the growth-regulator. A complete series of treatments was applied to an early lot of seedlings, lifted and treated on March 15 and 16, 1944, and to a late lot lifted and treated on April 22. The stock was 1-year-old eastern red oak and 3-year-old red pine. For each species on each treatment date, twenty uniform bundles of twenty-five seedlings each were assembled.

The emulsions, made as before with 5 gm. of lanolin per liter of water, carried naphthaleneacetic acid in the following concentrations: 0, 10, 20, 40, 80, 160, 320, and 640 mg./l. An additional emulsion carrying 80 mg. of naphthaleneacetic acid per liter was made up, using 5 gm./l. of a commercial wax[4] in place of lanolin; this was for comparison of carrier effects. After the growth-regulator crystals were dissolved in 95% ethyl alcohol, the solution was mixed into the wax emulsion. The commercial wax, though containing an emulsifier, flocculated on contact with the alcohol and, like lanolin, required 10 gm./l. of monoethanolamine stearate to bring about good dispersion. A set of seedlings not treated with growth-regulator served as controls. Thus there were ten treatments in all; these were twice replicated for each of the two species for each date, making eighty treatment units (bundles); at twenty-five per bundle, the total number of seedlings used was 2000.

Treatment consisted of complete immersion of bundles in the emulsions, with gentle agitation to assure full coverage, after which they were removed and allowed to drain. The bundles of seedlings were then packed upright in boxes, with moist sphagnum covering the roots, and were placed in common storage the same day. The following temperatures prevailed in the storage house: March 16, 51°; April 8, 51°; April 12, 51°; April 22, 53°; May 10, 60°; May 22, 65°; and May 23, 67° F.

On May 23 and 24 all seedlings were removed from storage and planted in a manner similar to that described for black locust and loblolly pine. Each of the two blocks in the plantation was divided into two plots to which the species were assigned at random. Within plots the twenty treatment-date combinations were randomized by rows.

In comparing this experiment with experiment I, it should be noted that, although in basic design both entailed two lengths of storage period, that factor was varied at opposite ends: in the second experiment two lifting dates were employed, with planting dates in common; in experiment I two planting dates were employed, with lifting dates in common. This difference provided opportunity to determine whether the major effects of the growth-regulator occurred in the early part or toward the end of the storage period.

RESULTS

EASTERN RED OAK.—On May 31, 8 days after planting, the following records were made of the seedlings: (*a*) number dormant, (*b*) number with some fully developed leaves, and (*c*) the total length of all shoots per seedling. Each of these criteria served as a rough measure of the inhibiting effects of naphthaleneacetic acid concentrations on shoot development.

Treatment date definitely influenced both the numbers of dormant seedlings

[4] The commercial wax was the proprietary product, Dowax, manufactured by the Dow Chemical Company, Midland, Mich. It contains a paraffin wax, bentonite, an emulsifying agent, and possibly other substances. So far as is known, its exact composition has not been published.

and the total shoot lengths. The mean percentage of seedlings dormant in the lot treated March 16 was 7.8; in the April 22 lot, 20.4. Similarly, the mean total lengths of shoots for the two treatment dates were 6.4 and 4.7 inches, respectively. Both differences were significant at the 1% level. These results appear to have been induced by two factors: (*a*) the direct effects of date at which the stock went into storage and (*b*) the increasing sensitivity of the plants to growth-regulator with approach to the date for normal revival of spring growth. Date of treatment had no significant effect on initial leaf development.

The effects of the various concentrations of naphthaleneacetic acid on growth, as indicated 8 days after planting, and on survival 1 year later, are summarized, with treatment dates combined, in table 4. The two highest concentrations definitely delayed break of dormancy and inhibited growth (fig. 3) according to all criteria listed; differences from untreated controls were all highly significant statistically except for leaf development with the 320-mg. concentration. Effects of the intermediate and lower concentrations, except the 0 level, were somewhat erratic, with inhibitory tendencies generally showing in the data on leaf and shoot development but not in the data on dormancy. In fact, the dormancy data alone suggest stimulatory effects from the lower concentrations. Results with the lanolin-80 treatment were particularly suggestive of stimulation.

Survival 1 year after planting was somewhat better for all naphthaleneacetic acid treatments than for untreated controls, except for the 640-mg. concentration (table 4); for four treatments the increases in survival were significant. In view of their originally strong inhibiting effect, it is of interest that the two highest concentrations of the acid caused no appreciable mortality. Date of treatment had no significant effect on survival.

Treatment dates, however, did affect seedling vigor, at least in so far as vigor was expressed by foliage development.



FIG. 3.—Effect of naphthaleneacetic acid on 1-year-old eastern red oak seedlings held in common storage from April 22 to May 23, 1944: *left*, untreated seedlings; *right*, entire seedlings dipped before storage into 0.5% lanolin emulsion containing 640 mg. of acid per liter. Seedlings completely dormant when placed in storage. Note proliferation of new roots from treated stock. Photo taken May 23, 1944.

Seven weeks after planting, the number of vigorous leaves per seedling was significantly greater (at the 1% level) for the April 22 treatment than for the March 16 series, and 52 weeks after planting the same highly significant difference still prevailed. Thus the greater degree of inhibition in storage associated with the later treatment presaged a higher degree of vigor after planting that carried over at least into the second year. This accords with the generally accepted

principle that for best results seedlings should be dormant, or essentially so, when transplanted. The interesting point is that the difference in degree of dormancy associated with the two treatment dates resulted in persisting differences in vigor of growth, even though not registering in terms of survival. It lends emphasis to the frequently ignored fact that differences between treatments in planting experiments often cannot be evaluated adequately by merely recording survival or mortality.

TABLE 4

EFFECT OF PRE-STORAGE TREATMENTS WITH VARIOUS CONCENTRATIONS OF NAPHTHALENEACETIC ACID ON SHOOT DEVELOPMENT AND SURVIVAL AFTER PLANTING OF EASTERN RED OAK SEEDLINGS. BASIS: 100 SEEDLINGS PER MEAN (MARCH 15 AND APRIL 22 TREATMENTS COMBINED)

TYPE OF EMULSION	CONCENTRATION OF NAPHTHALENEACETIC ACID (MG./L.)	MAY 31, 1944			MAY 30, 1945, SURVIVAL (%)
		Completely dormant seedlings (%)	Seedlings with some fully developed leaves (%)	Mean total length of seedling shoots (in.)	
None	Untreated control	12.0	51.0	7.1	26.0
Lanolin	0	5.0	63.0	7.6	30.8
Lanolin	10	4.0	37.0	6.4	38.8
Lanolin	20	6.0	50.0	6.3	52.8*
Lanolin	40	5.0	46.0	6.1	48.0*
Lanolin	80	3.0†	67.0	6.2	42.0
Comm. wax	80	8.0	42.0	5.9‡	54.0*
Lanolin	160	10.0	41.0	5.2§	56.0*
Lanolin	320	31.0§	45.0	3.7§	38.0
Lanolin	640	57.0§	14.0§	1.4§	24.8

* Survival benefits significant at 5% level.
† Growth stimulation significant at 5% level.
‡ Growth inhibition significant at 5% level.
§ Growth inhibition significant at 1% level.

RED PINE.—Records were limited to a single tally of survival on June 30, 1944, because heavy mortality (untreated controls 59.2%) rendered further observations useless.

Treatment dates had a highly significant effect. The survival of seedlings treated on March 16 was 18.6%; of those treated on April 22, 7.6%. Thus, in final results, the effects of date of treatment were opposite for red pine and red oak. However, this species difference apparently is merely a reflection of differing degrees of sensitivity and not a basic difference in nature of the reaction to the chemical. The difference in sensitivity may, at least in part, be a matter of proximity to the date for normal breaking of dormancy rather than inherent species characteristics (i.e., red pine normally renews growth early in the spring whereas the oaks are relatively late). With the oak, treatment resulted in a beneficial degree of growth inhibition in storage and more vigorous growth after planting; with the pine, buds were elongating by the later date and in such a state of tenderness that effects of the chemical exceeded mere inhibition to the point of injury.

The effects of all naphthaleneacetic

acid treatments, except at 20 mg./l., were distinctly harmful to red pine, the three highest concentrations causing complete mortality.

Discussion

The principal objective in treating forest planting stock with growth-regulating substances before or in storage is to facilitate holding the stock dormant and without deterioration. Delaying the break of dormancy is a requisite of successful storage because, with routine forest planting practices, most species will not satisfactorily survive transplanting unless the stock is either fully dormant or but little advanced beyond that state. Though survival is the first criterion of success in planting, subsequent growth and vigor also are important considerations that may be influenced by storage treatments or conditions.

In this study involving only unrefrigerated storage, both storage periods and naphthaleneacetic acid treatments affected seedling survival and growth, but the effects of storage periods were generally more pronounced. Black locust and loblolly pine deteriorated considerably in storage, especially during the last 2 weeks of a storage period extending into the warm weather of late May. Inasmuch as storage periods for these two species were varied only through different termination dates (not beginning dates), the tests with them unfortunately involved some confounding of effects of date of planting with the deteriorative effects of storage. However, even freshly lifted locust obviously could not have survived much better than the locust stock of the shorter storage period, since the latter averaged over 90% survival in an abnormally dry summer. The formation of large numbers of spindly shoots in the late-planted lot, and its much poorer survival (76%) and growth must be charged, therefore, largely against deterioration in storage. Freshly lifted loblolly pine from the same lot as used in this study, planted in an adjacent block on April 14, showed an average survival of 54% at the end of the first growing season. Corresponding values for the stored stock in the present test were 50 and 24%, respectively, for the shorter and longer periods. Thus practically all the deterioration in loblolly pine, as in locust, apparently occurred during the last 2 weeks of storage, when temperatures in the storage room ranged between 60° and 67° F.

Effect of length of storage period can be evaluated somewhat better for red pine and red oak, since with these species there was no confounding with planting date. Particularly for oak, growth in storage tended to be less and subsequent field performance better with shorter than with longer storage. However, inasmuch as seedlings of these species were treated on the different dates on which they were put into storage, some confounding of effects again was introduced between storage periods and condition of stock when treated. In general, the latter factor seems to have been more important than length of storage. Both species had become more sensitive to the acid by the time of the later treatments on April 22. The pine buds were swelling and were so tender that the seedlings suffered considerable injury, resulting in higher mortality after planting than was shown in the series treated earlier. The red oak, on the other hand, had by the later date become more sensitive only to the extent that the treatments were more effective in inhibiting subsequent growth in storage, and, in consequence, displayed greater vigor after planting in the field. The difference in

reaction to treatment was not great enough to affect survival.

Response to naphthaleneacetic acid varied with species, ranging from definite injury to significant benefits. The pines were generally more susceptible to injury than the hardwoods. Practically all emulsion-dip treatments, in particular, were injurious to both loblolly and red pines. The fact that, with the pines, foliage as well as woody portions were treated probably accounts in part for the differences in severity of effects between the two types of stock. These findings are in general agreement with those of OSTROM (6), who found that growth-regulator treatments effectively retarded break of dormancy of white ash held in unrefrigerated storage but lowered the survival of loblolly pine.

MARTH (5) found vapor treatments at 0.3 and 0.4 mg./cu. ft. and sprays at 0.01% (100 mg./l.) to be the most effective concentrations for delaying the break of dormancy in roses. In the present study vapor applications of naphthaleneacetic acid at 0.4 mg./cu. ft. were significantly beneficial and emulsion-dip applications at 100 mg./l. were significantly harmful to loblolly pine. However, red oak, after emulsion-dip applications of 20, 40, 80, and 160 mg./l., showed significantly higher survival than the controls; even with the 640 mg./l. treatment survival was not significantly lowered. With red pine, on the other hand, all emulsion-dips except those carrying 0 and 20 mg./l. of growth-regulator were significantly injurious.

These experiments obviously are not adequate as a basis for making practical prescriptions for nurserymen. They do, however, indicate that, with perfection of details of application technique and timing for different species, pre-storage treatment of stock with growth-regulators has possibilities for practical use. They demonstrate the existence of marked differences in sensitivity to naphthaleneacetic acid and in vulnerability to injury by it, as between the pines and hardwoods tested; these differences between the two taxonomic groups are believed to hold generally for other species. The experiments have reasonably well established the range of concentrations of chemical within which desired effects are most likely to be obtained and have shown something of the relative merits of different methods of application. The emulsion-dip method, for instance, which required the least in special apparatus, apparently is acceptable for treating hardwoods but is unsuited for use with pines. On the other hand, vapor and aerosol methods can be used for both classes of stock and, where facilities are available, probably are to be preferred. They permit more precise control over intensity of treatment than dips; in the latter, intensity is contingent upon the amount of chemical adhering to the plant and the length of time its potency is retained.

The marked stimulation of growth of new roots in red oak during storage (fig. 3)—and similarly of white ash (6)—suggests that the methods here employed for applying growth regulators might prove superior to conventional root-soaking treatments as a pre-planting measure apart from storage. The increased formation of root primordia resulting from treatment should materially improve chances for establishment of newly planted seedlings. Such treatment would necessitate proper timing so that, if storage were involved, planting would be finished before new roots had made enough growth to be susceptible to breakage.

Summary

1. Experiments involving methods of application and various concentrations of naphthaleneacetic acid, applied as pre-storage treatments to prolong dormancy and lessen deterioration of forest planting stock in unrefrigerated storage, were carried out on two hardwood and two pine species. Black locust and loblolly pine were treated with medium and relatively high concentrations of the chemical by three methods: exposure in a closed chamber to vaporized acid, to the acid dispersed in an aerosol mist, and by dipping the plants into lanolin emulsion in which the acid was dispersed. Eastern red oak and red pine were used in a test of eight different concentrations of the acid, applied only by the emulsion-dip method. Following 5–10 weeks' storage, the stock was planted in the field for observations on survival and vigor of growth.

2. Response to the treatments varied widely by species, ranging from significant benefits to distinct injury. The pines were much more vulnerable to injury than the hardwoods.

3. Main effects evident upon removal of the stock from storage were as follows: (*a*) With black locust, shoot elongation in storage was inhibited by both medium and high concentrations of the acid in all methods of application. (*b*) With loblolly pine, shoot elongation was inhibited by the higher concentration of acid applied in vapor form. Effects of other methods and concentrations on shoot elongation were either insignificant or erratic. However, the percentage of seedlings displaying apparently vigorous buds about a week after planting was increased by both levels of vapor treatment, by the lower level of acid in aerosol, and by aerosol alone, whereas the acid-emulsion treatments resulted in definitely lessened bud vigor. (*c*) With eastern red oak, shoot elongation was inhibited by all emulsions carrying 160 mg. or more of acid per liter. Concentrations of 80 mg. or less tended to hasten the break of dormancy slightly but did not result in any greater average shoot length than that displayed by untreated controls. (*d*) With red pine, shoots were not inhibited except as a corollary of injury.

4. Main effects evident after outplanting the stock in the field were: (*a*) With black locust survival was not affected by the chemical treatments, but vigor and survival of spindly shoots developed in storage generally were greater in treated seedlings than in controls. (*b*) With loblolly pine survival was generally improved by treatment with the acid in vapor form and at the lower concentration in aerosol, but only with the lower concentration of vapor was the increase statistically significant. Both acid-emulsion treatments were injurious, resulting in greater mortality than among controls and in retarded growth of survivors. However, the early indications of enhanced bud vigor in the vapor and aerosol series were not evident in greater height growth after planting. (*c*) With red oak survival was generally better for all levels of acid-emulsion treatment except the highest, which fell slightly below controls. For the 20, 40, 80, and 160 mg./l. concentrations of acid, the increase in survival was statistically significant. No differences in vigor due to chemical treatments were apparent. (*d*) With red pine survival was significantly less than for controls with all treatments except 20 mg./l. and lower concentrations of acid, which caused no marked reaction.

5. In general, all species exhibited significantly more growth and greater sur-

vival when planted after shorter (5–6 weeks) than after longer (8–10 weeks) periods of storage. For red oak, by exception, survival was not appreciably affected by storage period, though in vigor of growth this species concurred with the general trend. Even with the most effective treatments, dormancy was not adequately maintained over the longer periods, and some additional deterioration apart from breaking of dormancy apparently occurred.

6. These preliminary experiments indicate that, with additional experience and improvements in treatment techniques, naphthaleneacetic acid and probably other growth-regulator chemicals may have practical value for prolonging the period over which stock can be held in common storage without loss. Present results, however, offer no promise that growth-regulators can supplant refrigeration when storage for more than 1 or 2 months is required.

LITERATURE CITED

1. ENGSTROM, H. E., and STOECKLER, J. H. Nursery practice for trees and shrubs suitable for planting on the prairie-plains. U.S. Dept. Agr. Misc. Publ. 434. 1941.
2. HAMNER, C. L.; SCHOMER, H. A.; and GOODHUE, L. D. Aerosol, a new method of applying growth-regulators to plants. Science **99**: 85. 1944.
3. LAURIE, ALEX, and CHADWICK, L. C. The modern nursery, a guide to plant propagation, culture and handling. New York. 1931.
4. MAKI, T. E.; MARSHALL, HUBERT; and OSTROM, CARL E. Effects of naphthaleneacetic-acid sprays on the development and drought resistance of pine seedlings. BOT. GAZ. **107**: 297–312. 1946.
5. MARTH, PAUL C. Effects of growth-regulating substances on shoot development of roses during common storage. BOT. GAZ. **104**: 26–49. 1942.
6. OSTROM, CARL E. Effects of plant-growth regulators on shoot development and field survival of forest-tree seedlings. BOT. GAZ. **107**: 139–183. 1945.
7. TOUMEY, JAMES W., and KORSTIAN, CLARENCE F. Seeding and planting in the practice of forestry. Wiley, New York. 1942.

VOLUMETRIC STUDIES OF SEEDS, ENDOSPERMS, AND EMBRYOS IN PINUS PONDEROSA DURING EMBRYONIC DIFFERENTIATION

JOHN T. BUCHHOLZ

Introduction

Extensive investigations on the seeds of *Pinus ponderosa* during their development were undertaken in 1942 in order to obtain more accurate information concerning size relationships in the normal schedule of embryonic development. It was believed that a comparison of the embryogeny in large versus small seeds might shed some light on the function of the suspensor, since large seeds would provide more space for the elongation of this member. Such a study could also show whether morphological and anatomical differences exist between developing embryos of seeds of various sizes.

In mature ponderosa pine seeds the embryo represents about 10% of the whole seed volume and approximately 5% of the weight. However, at the stage during which the stem tip and cotyledons are differentiated, the embryo constitutes less than 0.5% of the volume of the seed. Thus the question arises: Does the young embryo begin to differentiate its organs at a size which is more or less constant—

an embryo of fixed volume—or does it differentiate only at a size which is closely related in some proportion to the size of the endosperm and seed?

The pine seed reaches its full size about the time of fertilization, which is more than a month earlier than the stages that are concerned with differentiation of the embryo. The changes involved during hardening of the seed coat do not increase the seed size. The seed in which fertilization failed to take place, or in which the embryo subsequently died, remains fully as large as a normal seed.

The trees which furnished the cones were all open wind-pollinated forest trees. Some ovules contain embryos resulting from self-pollination; others, from cross-pollination. Cross-pollination might represent combinations of diverse biotypes found within the species. On the whole, it is to be expected that the net result from wind pollination would tend to maintain the population in heterozygous condition, for even self-pollination in any particular seed would be likely to combine one of a variety of pairs of gametes in the zygote.

There is the possibility that differences in the embryos among seeds of a given size might be due to heredity. However, differences in heredity may be conditioned by the internal environment within the seed. Thus, if the mean sizes of the embryos are computed for each size class of seed, individual hereditary differences would tend to cancel out, and a close approach to the net effect of the internal environment of the seed could be obtained from the means of sufficiently large frequencies in each class.

Material and methods

The details concerning source, method of collection, and cone size, together with a record of the mean sizes of the embryos of various stages dissected from seeds of various sizes, have been given elsewhere (4). The present study is a continuation of this type of investigation on one of these trees more intensively and with several refinements of method.

In order to obtain embryos in all stages in the seeds of nearly all available sizes, collections from tree 3-38[1] were made separately on August 5, 8, and 12, approximately 6 weeks before seed maturation. From this tree, on which the present data are based, over 1100 seeds were obtained. None of the smallest-sized class of seeds (no. 11) contained embryos. The classes of seed size are given in the earlier paper (4). More than 150 of the seeds that appeared sound had shriveled contents.

In the present investigation, the volumes of the seeds, after sorting by sieves (4), were measured by liquid displacement before dissection. Similarly, following seed-coat removal, the volume of the endosperm was measured for each individual seed by liquid displacement in a capillary tube. Finally, the embryos were dissected out and their volumes were computed from measurements of embryo length and diameter, as defined in earlier papers (3, 4), by the formula $(\pi d^2/4)l$. Embryos of stages VI–VIII (see 3 for definition of stages) were measured for total length and for length of the embryo axes ending in the plumule primordium. The latter could be done by breaking away several cotyledons to expose the shoot tip to view.

The seeds of the most frequent size classes were not all dissected. These groups had been most thoroughly sampled, and continuation of dissection was

[1] The designation 3-38 refers to the tree P-Eld-3-38 of the Institute of Forest Genetics in Placerville, Calif.

deemed unnecessary. However, the 632 embryos which were dissected out constitute a population three times as large as any of the samples previously obtained. Embryos in all important stages from seeds of varying sizes were thus provided. Upon dissection, the stage of development of the largest embryo in each seed was noted, as well as that of the second largest, and the number of cotyledons was recorded.

Investigation

The duration of embryonic selection in the pine extends for more than a month. During this period of development the only change that may be recognized in the embryo complex is a very gradual increase in the size of the embryos, especially of the largest one, which usually assumes a dominant position at the forward end of the group.

The embryos are only slightly developed during this time of competition. There is a polarity indicated by the differentiation between the suspensor system and the meristematic cylinder borne on the end of the suspensor in each embryonic unit. The suspensors are derived from the elongation of successive layers of cells cut off previously by the embryos. Thus each embryo spins out its own suspensor which continually subtracts cells from the increasing volume of meristematic cells of the embryonic cylinder. The embryo adds unicellular elements (embryonal tubes) to the suspensor at first. Later the multicellular secondary suspensor (of numerous parallel embryonal tubes) is elaborated. Thus the struggle between embryos becomes one of competition in the rate of growth due (*a*) to elongation of suspensor tissue and (*b*) to meristematic increase of cells in the respective embryos.

About 2 weeks after fertilization an embryo emerges from the complex in a dominant position. Its cylinder of meristem enlarges gradually despite rapid development of its suspensor. The twists and coils appearing in the suspensor complex dislocate the orientation of embryos in secondary positions. Snags or spurs of embryonal tubes belonging to the suspensor of the largest embryo ensnare smaller embryos and push them back toward the micropyle. In this way, about 5 weeks after fertilization, the largest embryo—usually in terminal position in the complex—begins its differentiation (fig. 1).

The embryos that are left behind in embryonic selection within each seed usually fail to develop beyond this stage. Most of them remain much less developed. The data of this study indicate that the second largest embryo in a seed rarely exceeds stage II in size, even when it persists to seed maturity.

Differentiation of embryo

After the period of embryonic selection, differentiation of the dominant embryo progresses with considerable rapidity. Although internal differentiation occurs earlier, there is no outward criterion until stage III is reached, when the primordium of the shoot tip appears (figs. 1, 2). The primordium is a special meristematic area at the tip of the larger embryonic cylinder. In dissected embryos the plumule primordium is a very conspicuous area which appears more translucent, probably owing to the character of the vacuoles and other cell contents and to the lack of intercellular air spaces within this tissue.

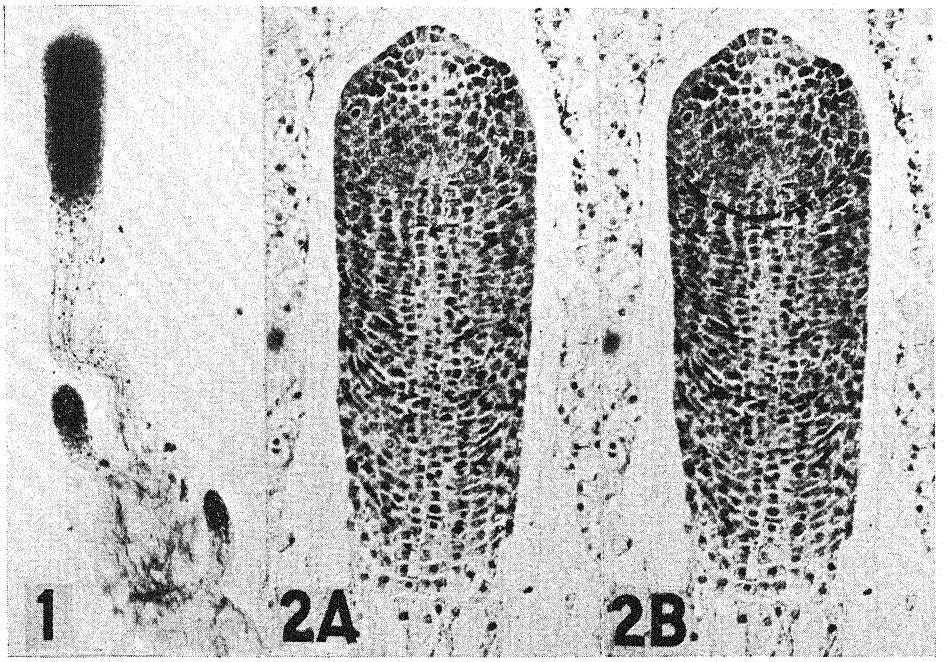
The embryos in seeds within the same cone or other cones of a given tree will not be found in the same stage of development 5 weeks after fertilization. Those whose growth has been most rapid may

be in an advanced stage of development, with their cotyledons already formed and well elongated, while others may still fail to show the shoot-tip primordium. Chronologically, they are of the same age, since fertilization takes place in all ovules of a cone within a few days. It was necessary, therefore, to define a series of morphological growth stages for the embryo which were designated by the Roman numerals I–VIII. These stages were described elsewhere (3). It may be recalled here that the origin of the plumule apex occurs in stage III (see figs. 1, 2) and that the cotyledonary primordia arise in stage IV (figs. 3, 4). Figures 5 and 6 show stage V.

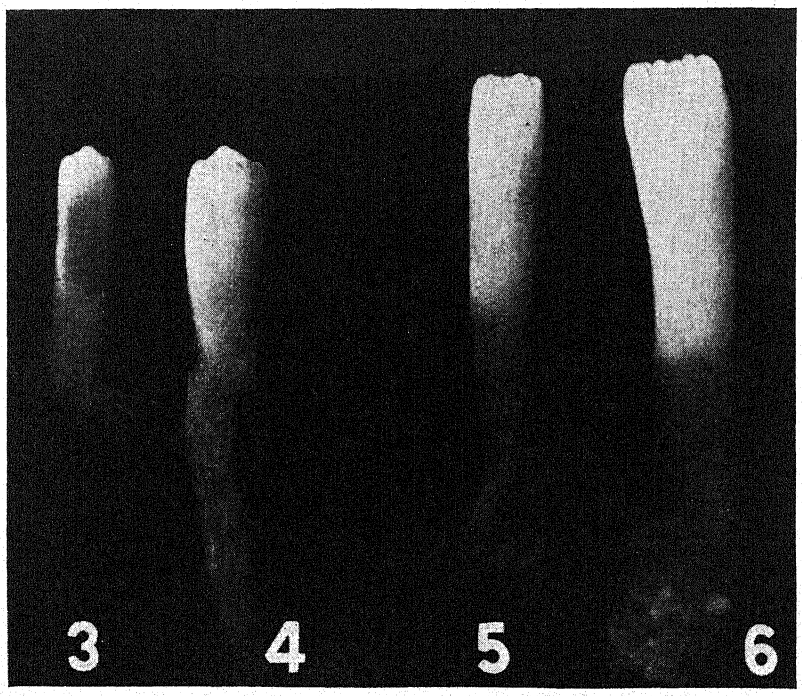
Duration of stages

The time intervals between stages have been calculated in the embryos of a few trees of *P. ponderosa*. The rapidity of growth was quite marked. Two collections were made 4 days apart from tree 3-14 and were used in an analysis that would indicate the duration of the stages. Seventy-eight per cent of the embryos were in stages I–IV on August 1 and only 30% on August 5; 22% were in stages V–VIII on August 1 and 70% on August 5. Table 1 indicates the frequencies and percentages of embryos in different stages on these two dates.

With the aid of table 2, it is possible, using the percentages given in table 1, to



Figs. 1–2.—Fig. 1, dissected embryonic system of *Pinus banksiana*, showing dominant embryo advanced to stage III while other embryo units are being suppressed. ×25. Fig. 2*A*, *B*, longitudinal sections of pine embryo (*Pinus banksiana*) in stage III, at the time when shoot tip is differentiated. Note internal differentiation of small spherical meristematic mass (marked in 2*B*), from which will develop shoot and root of mature embryo. Larger portion below is root cap and suspensor. ×80.



FIGS. 3–6.—Dissected embryos of *Pinus ponderosa*. ×15. Fig. 3, stage IV from no. 13 seed with endosperm volume of 20 cu. mm. Relatively small plumule primordium surrounded by eight cotyledons. Fig. 4, stage IV from large no. 18 seed with endosperm volume of 50 cu. mm. Relatively large stem-tip primordium surrounded by primordia of fourteen cotyledons. Larger throughout than embryo in figure 3. Fig. 5, stage V from no. 13 seed with endosperm volume of 24 cu. mm. The seven cotyledons equal in length to plumule primordium. Fig. 6, stage V from no. 17 seed with endosperm volume of 45 cu. mm. Large stem tip now obscured by thirteen cotyledons of approximately its length. Smaller embryos eliminated in embryonic selection may be seen below, where they were pushed back by massive suspensor of large embryo.

TABLE 1

AMOUNTS AND PERCENTAGES OF EMBRYOS IN DIFFERENT STAGES OF DEVELOPMENT ON AUGUST 1 AND 5 (TREE 3-14)

DATE OF COLLECTION	STAGE								TOTAL
	I	II	III	IV	V	VI	VII	VIII	
August 1 (2 cones):									
Frequency	1	13	17	9	9	0	2	0	51
%	*2*	*25*	*33*	*18*	*18*	*0*	*4*	*0*	*100*
August 5 (5 cones):									
Frequency	4	9	7	31	28	44	26	18	167
%	*2*	*5*	*4*	*19*	*17*	*26*	*16*	*11*	*100*
Total embryos (7 cones)	5	22	24	40	27	44	28	18	218

estimate the rate of progress in growth stages passed through during the 4-day interval. Thus, the 2% of the embryos in stage I and one-fifth of the 25% of the embryos in stage II on August 1 remained unchanged on August 5, indicating that these are stages of long duration. The following calculation was used to derive the approximate rate of change in stage per embryo:

TABLE 2

PERCENTAGE CHANGE IN STAGE DURING FOUR-DAY INTERVAL

DATE OF COLLECTION	STAGE I	II	III	IV	V	VI	VII	VIII	TOTAL
AUGUST 1	2	25	33	18	18	0	4	0	100
ESTIMATED SHIFTS THROUGH 3, 2, 1, OR 0 STAGES	2	5	4	3 16	13 17	11 13	4 5	7	
AUGUST 5	2	5	4	19	17	26	16	11	100

Growth changes through three stages:

$7 + 5 + 13 = 25$ embryos $\times 3 = 75$ changes in stage.

Growth changes through two stages:

$11 + 13 + 17 + 16 = 57$ embryos $\times 2 = 114$ changes in stage.

Growth changes through one stage:

$4 + 3 + 4 = 11$ embryos $\times 1 = 11$ changes in stage.

No change in stage:

$2 + 5 = 7$ embryos $\times 0 = 0$ change in stage.

Total 100 embryos with 200 changes in stage in 4 days,

or 100 embryos with 50 changes in stage in 1 day,

or $\frac{1}{2}$ change of stage per embryo per day.

It is possible that the stages are passed through more rapidly than the above rate. The seeds of another tree, 3-57, were collected at an interval of only 1 day. This tree had relatively fewer embryos in stages I and II; hence the rate of change of stage was more rapid, being more nearly two-thirds stage per day. The seeds of tree 3-38 were collected on August 5, 8, and 12, but the seeds of August 5 were not all kept accurately separated from those collected on August 8. However, it was possible to use the data by adding together the seeds of August 5 and 8 and comparing them with the collection of August 12, some of which were dissected on August 13. The mean time between collections was 6–6½ days, giving a rate of 0.47 stage per day for a 6½-day period and 0.52 stage per day for a 6-day interval.

The large number of seeds from tree 3-38 made it possible to compare the intervals between embryonic stages separately in seeds of different sizes. It was found that the rate of change in stage was 0.57 stage per day for no. 17 seeds, 0.54 stage per day for no. 16 seeds, 0.62 stage per day for no. 15 seeds, 0.42 stage per day for no. 14 seeds, and only 0.35 stage per day for no. 13 seeds. Thus it appears that the embryos of seeds of

average size (no. 15) grow most rapidly and that the embryos in the smallest seeds develop much more slowly. The slower rate of growth in the largest seeds might be due to the greater expenditure of tissue in suspensor formation and to the fact that the embryos must grow larger before differentiation. It should be noted that embryos of the largest seeds differentiate only after the embryonic cylinder has become considerably larger than in those of average size (compare fig. 3 with fig. 4 and fig. 5 with fig. 6).

There are evidently some variations in the intervals between embryonic stages, but an interval of 2 days (at the rate of 0.5 stage per day) is used for convenience in discussion in the following pages.

Embryo size in relation to size of endosperm and seed

Table 3 shows results of measurements made on embryos, endosperm, and seeds and includes all stages that were dissected from twelve cones of tree 3-38. It is clearly indicated that the embryos of large seeds, at the time of stem-tip and cotyledon differentiation, are much larger than those of small seeds.

In order to test the volumetric relations of embryos in each stage against the

TABLE 3

EMBRYO LENGTH AND DIAMETER IN RELATION TO SIZE OF SEED, VOLUME OF ENDOSPERM, AND STAGE OF DEVELOPMENT FOR 632 SEEDS*

Seed class	Endosperm (vol. cc.)	Stage I			Stage II			Stage III			Stage IV			Stage V		
		f	*d*	*l*	*f*	*d*	*l*	*f*	*d*	*l*	*f*	*d*	*l*	*f*	*d*	*l*
12	0.018	2	206	270	2	352	614	4	308	738	4	582	1444	1	775	1906
13	.023	11	232	333	9	377	599	7	457	911	16	624	1421	6	768	1948
14	.029	10	252	378	8	375	697	22	466	958	30	629	1463	6	770	1891
15	.035	19	249	346	23	396	634	27	497	983	54	667	1471	17	838	2042
16	.040	9	245	388	10	403	654	14	536	1017	19	682	1480	22	851	2161
17	.046	8	251	346	15	431	699	13	528	1089	16	715	1632	14	922	2132
18	0.052	...	....	.....	...	....	.....	4	620	1250	5	746	1659	1	946	2294
Mean total	.....	59	245	353	67	400	655	91	498	993	144	662	1492	67	847	2080

TABLE 3—*Continued*

Seed class	Endosperm (vol. cc.)	Stage VI				Stage VII				Stage VIII			
		f	*d*	*tot.l*	*ax.l*	*f*	*d*	*tot.l*	*ax.l*	*f*	*d*	*tot.l*	*ax.l*
13	0.023	4	798	2352	2239	...	.....	.....	.....	...	.....	.....	.....
14	.029	2	876	2596	2426	...	.....	.....	.....	...	.....	.....	.....
15	.035	15	1023	3025	2813	13	1216	4249	3663	22	1446	5322	4232
16	.040	28	1050	3097	2860	11	1230	4394	3768	27	1448	5529	4342
17	.046	18	1114	3221	2969	12	1287	4408	3788	31	1548	5535	4409
18	0.052	2	1023	3054	2806	11	1316	4544	4041	8	1629	5725	4478
Mean total	.....	69	1040	3055	2827	47	1282	4391	3808	88	1475	5444	4370

* Diameter of embryo is *d*, frequency is *f*; *l* is length in first five stages. In last three stages, *ax.l* is length of axis alone, and *tot.l* is over-all length including cotyledons; lengths and diameters are given in microns.

TABLE 4*

RELATIONSHIP OF EMBRYO SIZE (VOLUME) TO SEED VOLUME AND ENDOSPERM VOLUME DURING EXTERNAL DIFFERENTIATION†

SEED SIZE		STAGE III					STAGE IV					STAGE V					STAGE VI					END. VOL. COMPUTED AS 36% OF SEED VOL.
Class	Vol. cu. mm.	*f*	Vol. end. cu. mm.	Vol. emb. cu. mm.	Emb. / Seed (%)	Emb. / End. (%)	*f*	Vol. end. cu. mm.	Vol. emb. cu. mm.	Emb. / Seed (%)	Emb. / End. (%)	*f*	Vol. end. cu. mm.	Vol. emb. cu. mm.	Emb. / Seed (%)	Emb. / End. (%)	*f*	Vol. end. cu. mm.	Vol. emb. cu. mm.	Emb. / Seed (%)	Emb. / End. (%)	
12.....	50	4	16	0.092	0.18	0.57	4	21	0.384	0.78	1.83	1	19	0.899	1.80	4.73	....	....	.....	.....	.....	18
13.....	64	7	27	.149	.23	.55	16	24	.437	.68	1.82	6	24	0.902	1.41	3.76	4	24	1.176	1.84	4.89	23
14.....	82	22	29	.163	.20	.56	30	27	.455	.56	1.68	6	28	0.882	1.08	3.12	2	28	1.565	1.91	5.58	29
15.....	97	27	36	.176	.18	.49	54	33	.514	.53	1.56	17	34	1.126	1.17	3.30	15	35	2.486	2.57	7.11	35
16.....	113	14	29	.229	.20	.59	19	36	.451	.40	1.25	22	35	1.229	1.09	3.63	28	35	2.682	2.38	7.65	41
17.....	126	13	47	.238	.19	.51	16	43	.655	.52	1.52	14	42	1.423	1.13	3.48	18	44	3.048	2.42	6.90	45
18.....	140	4	49	0.377	0.27	0.77	5	48	0.596	0.42	1.20	1	50	1.612	1.15	3.22	2	50	2.510	1.79	5.00	50
Mean.....		7	....	0.203	0.21	0.58	7	....	0.499	0.56	1.55	7	....	1.152	1.28	3.61	6	....	2.244	2.17	6.21	.....

* In obtaining volumes for this table by liquid displacement, an experimental error up to 8 cu. mm. may have been introduced by the estimation of meniscus level between wide graduations. Thus there was considerable fluctuation in the ratio of endosperm volume to seed volume. The mean value obtained for the endosperm volume was 36% of the seed volume. This figure was used to establish a computed volume of the endosperm in each size class—the column at the extreme right.

† Frequency is designated by *f*. Embryo, endosperm, and volume are abbreviated. See text for further explanation.

volumes of endosperms and seeds, the mean volumes of the embryo were computed for the four stages which involve stem-tip and cotyledon formation. These are shown in table 4. The best test that could be devised which might show a constancy of volumetric relations was that of computing the ratio of volume of embryo to volumes of endosperm and of seed. These values, in percentages, are entered in the appropriate columns of table 4.

The embryos have a closely constant ratio to the size of seed and of endosperm during the stages in which shoot tip (stage III) and cotyledons (stage IV) first appear and begin to elongate. During stage III the embryo constitutes only 0.2% of the volume of the seed and 0.6% of the volume of the endosperm. Errors introduced by use of the formula for a cylinder, as described above, could be partly overcome by making corrections for the domed curvature of the shoot tip, for irregularities due to cotyledons, and for taper below, but these corrections would be nearly constant and would not change the relative values.

It may be concluded that the volume of the embryo at the time of differentiation of its shoot tip (stage III) is closely related to the volume of the endosperm; it is also related to the volume of the seed. The larger embryos have larger shoot-tip primordia which seem to be proportional to the size of the embryo. The volume of the embryo at the time of initiation of cotyledonary primordia (stage IV) is also closely related to the volumes of the endosperm and seed. Although at this stage the ponderosa pine embryo has about two and a half times the volume it possessed at shoot-tip initiation, it still amounts to less than two-thirds of 1% of the volume of the seed and less than 2% of the endosperm volume.

TABLE 5

FREQUENCY OF COTYLEDON NUMBER IN EMBRYOS IN RELATION TO SEED SIZE

Seed size	Cotyledon number											
	6	7	8	9	10	11	12	13	14	15	16	Mean
12	.....	.....	2	1	.....	.....	.....	.....	.....	.....	.....	8.3
13	1	1	9	7	5	.....	.....	.....	.....	.....	.....	8.6
14	1	1	7	13	7	8	.....	.....	.....	.....	.....	9.3
15	.....	2	10	19	35	26	15	5	.....	.....	.....	10.2
16	.....	.....	4	15	16	32	34	6	1	.....	.....	10.9
17	.....	.....	1	8	10	28	25	16	2	.....	.....	11.4
18	.....	.....	.....	2	3	5	7	3	3	2	1	12.1

In embryos of all sizes the size of cells and of nuclei as seen in sections at the time of primordium initiation remains essentially the same. This is true in comparable parts of the embryos: root-cap cylinder, shoot-tip primordium, or cotyledonary primordia. A larger volume in the embryo represents a larger number of cells throughout all its parts, with the possible exception of the cotyledonary primordia (see below).

COTYLEDON NUMBERS

The frequencies of different cotyledon numbers in seeds of various sizes are presented in table 5. The fact that cotyledon counts from the smallest seeds are based

upon small numbers is unavoidable, since these sizes of seeds were least frequent in the collections. In addition, because of the slower rate of development in these seeds, few were found at the stage of cotyledon initiation.

A correlation between seed size, embryo size, and number of cotyledons developed may be clearly seen from the data. This corroborates the results obtained earlier (4) from a much smaller number of seeds. It might be supposed that there is more room for a large number of cotyledons on the shoulders of large embryos than on small ones. This would be expected if the plumule primordium of a large embryo were larger than in a small embryo and the cotyledonary primordia did not share proportionately in the increase in size of the embryo as a whole.

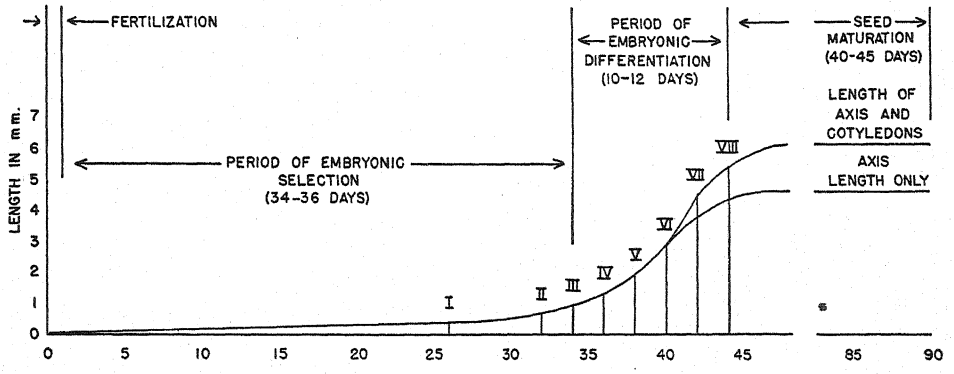


FIG. 7.—Mean growth-curve of embryos in axis length for period of fertilization to maturity of seeds.

GROWTH-CURVE OF EMBRYO

A diagram presenting an over-all picture of the entire program of development from fertilization to maturity (i.e., in growth in length) may be constructed from the data given. Figure 7 represents the average growth-curve for all sizes of embryos of tree 3-38.[2] In this curve the known items are the period from fertilization to stage IV (approximately 35 days), the mean axis lengths of the embryos in various stages, the 2-day mean interval between stages from stage III onward, and the mean axis length of the embryo in a group of mature seeds harvested from the tree 6 weeks later.

It is evident that for a period of about a month following fertilization—the period of embryonic selection—the embryos grow very slowly in diameter and length. After stage III is reached, the dominant embryos enlarge rapidly (figs. 3–6). From stage VIII onward to the maturity of the seed the rate of elongation subsides and is gradually arrested. Between stages VII and VIII the curve falls off sharply only when the axis length is plotted. If the total over-all mean lengths are plotted, the height of the curve continues to increase, owing to the addition of the later-developing cotyledonary meristem. It is clear that during the second portion of the period of development, which involves embryo differentiation, the curve of growth in axis length follows the usual sigmoid pattern.

[2] Since the smaller seeds had no embryos in the later stages of development (table 3), a discrepancy is introduced into these stages on the growth-curve; the basic trend of the curve, however, remains the same.

Discussion

The feature revealed by this and the previous study (4) which is most outstanding is the diversity of stages that may be found among embryos that are collected from a tree on a given date. The embryos are of the same chronological age; they result from fertilizations that take place on the same day or within a few days. However, they are found to diverge as much as 9 or 10 days in terms of the average embryos.

A genetic variation might account for the difference in stage among the dominant embryos of different seeds. The divergence of stages could, therefore, represent a measure of differences in inherent embryonic vigor. An embryo found in stage VIII 35 days after fertilization is 8 days in advance of the mean (then in stage IV). It is a precocious embryo from the standpoint of vigor of growth. An embryo from the same cone which is still in stage I is retarded 8 or more days behind the mean. It has an inferior performance record. The precocious embryo may be rated as growing at the rate of 43/35, or 123% of the average; the backward embryo as growing at the rate of 27/35, or 77% of the average in a given cone or tree. It is possible that these interseminal performance records may hold for the growth rates that are observed in the same individuals after germination of the seeds. Such differences in the vigor of seedlings would be worthy of special attention if they could be realized in cultures of nearly mature embryos. The extremes might seem to add up to the kind of differences in vigor that have been observed between the averages of hybrids and those of normal wind-pollinated progeny (3).

It was thought that this investigation might uncover data which would have a bearing on the function of the suspensor. There was never any doubt as to the importance of this organ as a weapon of competition during embryonic selection, which was pointed out previously (1, 2). Nothing was noted that would serve to alter this conclusion. It would appear that the superior embryo, which always has the largest suspensor, preserves a fine balance between meristematic activity in the embryonic cylinder and suspensor elongation.

When comparisons were made between large and small seeds, it was usually found that the embryos in small seeds were not only smaller in volume per stage but that they were more retarded in the stages which were found. Large seeds yield a greater diversity of stages on a given date, but the most precocious embryos are usually to be found in the medium- and large-sized seeds only. It should not be concluded, however, that all ponderosa pine seeds in the nos. 12–14 group have a slow rate of development. This holds only for trees in which those sizes are smaller than the average. In trees in which no. 13 seeds are the average, that size may have the same or even a more rapid rate of development than the average size (no. 15) in tree 3-38.

The completion of a growth-curve showing the development of the embryo from fertilization into the maturity of the seed has provided an over-all quantitative record of the process of seed development in pines and related conifers. Many features now become more clearly apparent.

The entire embryogeny falls into two periods. The early period, occupying from 4 to 5 weeks in medium and large seeds (longer in the smallest ones) is given over to embryonic selection. Numerous embryos, still more or less undifferentiated, are engaged in an intense in-

ternal "struggle for existence." Usually only a single embryo per seed survives this competition, bounding forth in a spurt of growth and differentiation which assures its success as the selected embryo of the seed. This occupies the second period of development, less than half as long as the first. It is not likely that the same mathematical equation would fit both regions of this curve of growth or that this would be required, since the processes of embryonic development are different in each period.

The first period may be absent in angiosperms which are not generally polyembryonic. If such a period of delay in growth is present in some angiosperms, it may be due to a competition between endosperm and embryo, both of which result from a fertilization process. The writer entertains some doubt that such a relatively prolonged period of slow growth would be evident in angiosperms even where polyembryony is found. It must be remembered that the polyembryony observed in angiosperms does not result in the decisive selection of a single embryo per seed but that usually several embryos are matured within a seed. In pines, in conifers generally, and in all gymnosperms, the usual condition (more than 98%) of polyembryony is terminated decisively in the selection of a single embryo in the seed.

The correlation between seed size and embryo size in ponderosa pine seems significant. The large seeds may offer a better opportunity to the embryo to express a capacity for rapid growth in a short time, while the more crowded condition within a small seed serves to intensify and prolong the period of embryonic selection. Thus, in larger seeds, where there is less crowding among the numerous embryos and where longer suspensors may be produced, embryonic selection may progress more rapidly and come to a decisive conclusion at an earlier date than in the smallest seeds.

However, it is possible that the ratio of size of embryo to size of seed might be explained on a basis other than spatial relations of embryonic development. The nutritional conditions in different regions of the cone may be variable. Hence differences in embryo development could be correlated with nutritional differences occurring in various portions of the cone. It is also possible that seeds vary in their permeability to food materials (according to seed-coat thickness or to the nature of the nucellar tissue) which might be conditioned in association with the factors determining seed size. These and other factors may be involved which are beyond the scope of this study to determine.

Using seed size as a determinant of embryo size, it is possible to speculate on the factors concerned in the establishment of seed size. It is usually assumed that the pollen grains which fall on the micropyle and grow in the ovule furnish the stimulation for ovule development and enlargement. This may be expressed by the liberation of hormones from the pollen grains and tubes. If so, then the stimulus provided may be expected to vary owing to genetic differences among the pollen grains. It may also vary quantitatively, depending upon the number of pollen grains which give rise to active male gametophytes.

There have been workers, on the other hand, who would relate the size of the ovule to other factors, independent of the pollen stimulus. BURLINGAME (5) found that ovule development in *Araucaria* seemed to occur independently of pollen effect. Ovule size may be related to the size of the cone on which it is borne, the position of its own scale with-

in the cone (6, 8), and the vigor of the tree. It may also be conditioned by the rate of development of the female gametophyte or by the hormones possibly furnished by that tissue or by the parent sporophyte.

It is generally known that a large seed gives rise to a larger seedling at the time of germination than a small seed. Some foresters have believed that this initial advantage is permanent. Others have contended that the larger seedlings are not necessarily the more vigorous individuals—that the initial advantage in size is only transitory. The latter belief has been borne out by the work of RIGHTER (7) and of the author (3) in an earlier paper. The discovery in pines that the embryos chalk up their own growth records through a series of easily recognized stages (if observed at appropriate intervals) is an important fact that has been hitherto neglected. It would seem to offer the basis for a new experimental approach to problems of seed selection and improvement.

Summary

1. Data were obtained on the seeds, endosperms, and embryos from cones of previously described trees of *Pinus ponderosa*. Volumetric measurements were made, cotyledons were counted for embryos from different sizes of seeds, and the rate of growth of the embryos was computed.

2. For a period of about 5 weeks there is little embryonic differentiation, the embryos within each seed being engaged in a competitive struggle for survival—a period of embryonic selection.

3. After the dominant embryo has been established, it spurts ahead in rapid growth and differentiates all its organs in a period of 10–12 days. Seed ripening occurs in a few weeks with little change in embryo size.

4. A great diversity of stages is found among embryos collected on a given date. This makes possible a computation of the presumable rate of growth between successive collections of seeds from the same tree.

5. The average rate of growth is about $\frac{1}{2}$ stage per day during embryo differentiation. It is much slower in the smallest seeds.

6. At the time of shoot-tip differentiation and of cotyledon initiation, the volume of the embryo is closely related to the volumes of endosperm and of seed.

7. There exists a correlation among seed size, embryo size, endosperm size, and number of cotyledons in the embryo.

DEPARTMENT OF BOTANY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

LITERATURE CITED

1. BUCHHOLZ, J. T. Suspensor and early embryo of *Pinus*. BOT. GAZ. 66:185–228. 1918.
2. ———. Developmental selection in vascular plants. BOT. GAZ. 73:249–286. 1922.
3. ———. Embryological aspects of hybrid vigor in pines. Science 102:135–142. 1945.
4. BUCHHOLZ, J. T., and STIEMERT, M. L. Development of seeds and embryos in *Pinus ponderosa*, with special reference to seed size. Trans. Ill. Acad. Sci. 38:27–50. 1945.
5. BURLINGAME, L. L. The morphology of *Araucaria brasiliensis*. II. The ovulate cone and female gametophyte. BOT. GAZ. 57:490–508. 1914.
6. MUNNS, E. N. Effect of location of seed upon germination. BOT. GAZ. 72:256–260. 1921.
7. RIGHTER, F. I. *Pinus:* The relation of seed size and seedling size to inherent vigor. Jour. For. 43:131–137. 1945.
8. WRIGHT, J. W. Influence of size and portion of cone on seed weight in eastern white pine. Jour. For. 43:817–819. 1945.

A CYTOLOGICAL STUDY OF UROMYCES ALOËS

M. J. THIRUMALACHAR

Introduction

Uromyces aloës (Cke.) Magn. has been found parasitizing as many as seven species of *Aloe* in Africa (11) and is known to occur in India on *Aloe vera* and *A. spicata*. Only the telial stage was described in the original description of the species; later, by a morphological and cultural study, AJREKAR and TONAPY (1) found the presence of pycnia. They also observed some very interesting features in the germination of the teliospores which invited further study. It was pointed out, for example, that the teliospore on germination puts out a promycelium into which the granular contents of the spore migrate. The germination was stated to be peculiar in that the basidium is two-celled (rarely three-celled) instead of the usual four-celled condition. The upper cell of this two-celled promycelium develops a germ tube and functions directly as the sporidium.

Experience with the cytology of rusts has indicated that the presence of a two-celled basidium is always correlated with some variation in the nuclear sequence. Further, the suppression of the development of sporidia and the consequent assumption by the upper cell of the promycelium of the function of sporidia, would, if confirmed, present us with an interesting variation not usually met with in rusts. This problem was kindly suggested to the writer by Professor S. L. AJREKAR, to whom the author is indebted for many valuable suggestions. On account of the scarcity of specimens, the work could not be undertaken readily. AJREKAR, who first collected the rust near Telegaon, Poona, was unable to collect material again in the same locality. Recently a collection was made by the writer near Kadur, Mysore State, which enabled a detailed cytological study. The present work adds cytological details and thus throws light on the life-cycle; it completely confirms the findings of AJREKAR and TONAPY.

METHODS.—Material was fixed in Karpechenko's modification of Navashin's fluid, and microtome sections 8–10 μ thick were cut and stained either with Heidenhain's iron-alum haematoxylin or with Newton's iodine gentian-violet, counterstained with light green. Teliospores were germinated by the method suggested by the writer (26). Gentian-violet and light-green combinations were used in most cases.

Observations

The rust is foliicolous and amphigenous, with a yellowish ground color interspersed with concentrically arranged erumpent sori containing chocolate-brown powdery spores. The spots often coalesce with one another and thus occupy a major portion of the leaf surface.

SPORE-FORMS

PYCNIA.—Pycnial initials are formed by the grouping of hyphae beneath the epidermis and are developed following sporidial infections. They are minute, orange-yellow, and concentrically grouped. Mature pycnia (fig. 1) are flask-shaped, flattened at the apex, and open out by a small pore formed by the rupture of the epidermis. There are no traces of any

ostiolar flexuous hyphae projecting beyond the pore. The pycniospores are extruded and collect in a tiny droplet of secreted nectar on the outer surface. The pycnial initials, the mycelium bearing the pycnia, and the pycniospores are all uninucleate.

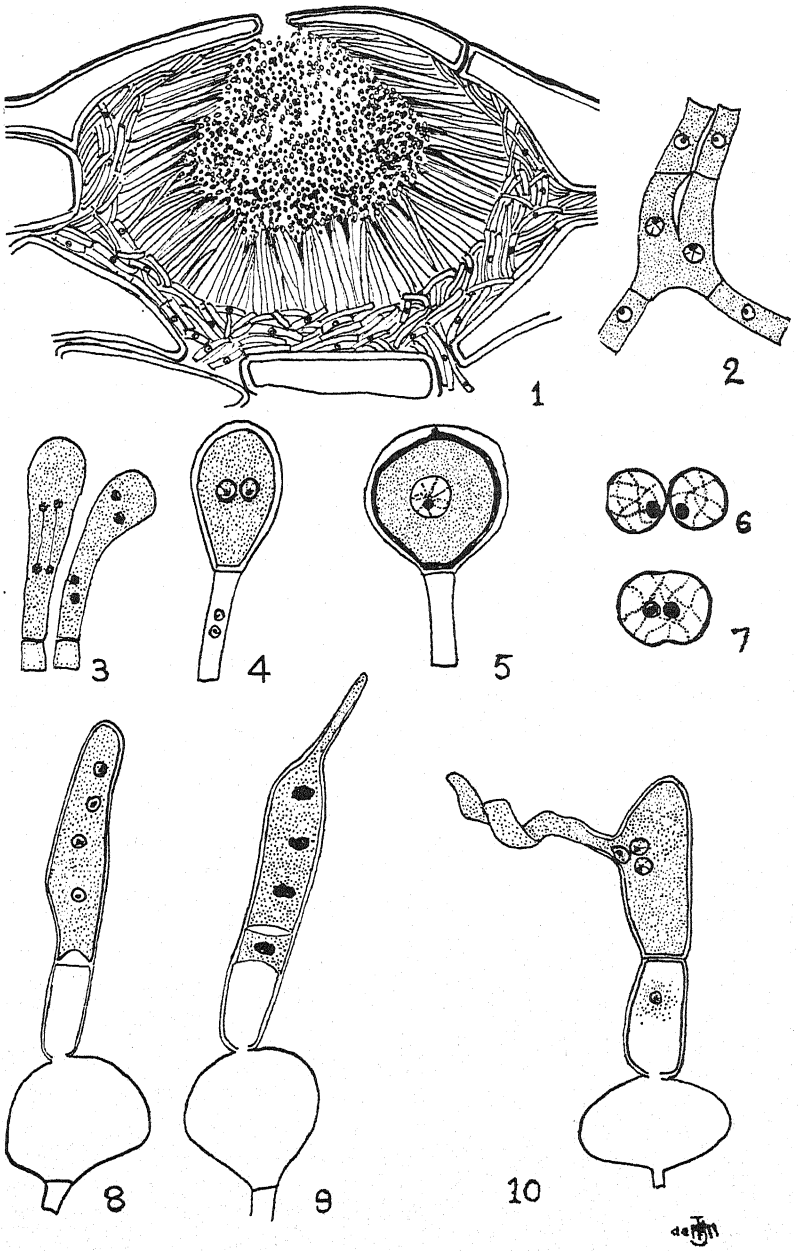
TELIA.—The telia are formed soon after the development of the pycnia. Macroscopically the telial initials appear on the surface of the infection patch as yellowish flattened pustules. These are sometimes formed directly after sporidial infections in an abbreviated cycle. Even after completion of the development of telia, the mycelium bordering the infection patch spreads toward the base of the leaf, developing new pycnia and telia. Since it does not reach the shoot growing-point of the host (which is at the base of the radical leaves), the rust does not become systemic.

Sections through a young telium show a plectenchymatic mass of hyphae grouped beneath the leaf epidermis. Cells of the hyphae are all uninucleate. Toward the center of the telium primordium and generally extending toward its periphery, cell fusions between neighboring cells take place. These are effected by dissolution of the separating wall, either partially or completely (fig. 2). Such types of cell fusions have been described in *Phragmidium speciosum* (7), in *Phragmotelium mysorensis* (27), and in *Thekopsora hydrangeae* (22) within the *aecial primordia*.

Following production of the fusion cells, the other plectenchyma cells begin to disintegrate. The binucleate fusion cells elongate in a vertical direction, and each cuts off a stalk cell and a teliospore (figs. 3, 4). The two nuclei within the pedicel remain small and inconspicuous and finally degenerate prior to the maturity of the teliospore. The wall of the spore thickens and turns yellowish-brown in color. The two nuclei fuse and form a single large fusion nucleus (fig. 5). Prior to fusion the two nuclei develop a characteristic structure found during the prophase of the mitotic division (figs. 6, 7). This has been referred to as the nucleus of the expanded type by SAVILE (25). In the early stages following nuclear fusion the two nucleoli remain distinctly separate but finally fuse. The mature spore is thick-walled, with a single large fusion nucleus (fig. 5) and an apical germ pore, which in some cases is laterally situated. The pedicels, which are very long—up to 130 μ—are fragile and break up just beneath the point of the hilum. Consequently, the mature teliospores are dispersed very much like the urediniospores of other rusts and impart a dusty, powdery appearance to the sorus.

BASIDIA.—The teliospores germinate readily without a period of rest being necessary. Prior to germination the spore coats slightly swell in water, thus revealing the distended germ pore. The promycelium emerges as a small papilla, gradually elongating in length and becoming cylindrical. The granular contents of the teliospore along with the fusion nucleus migrate into the promycelium. Unlike some of the rust species studied by SAVILE (25), the prophase stages of the nuclear divisions do not take place within the teliospore itself. As a result of two successive divisions of the fusion nucleus, four free nuclei are derived and lie in a linear row (fig. 8). Further development of the basidium is uniformly consistent in a majority of the cases, and only a few variations have been observed.

In the normal type of development, after the formation of four free nuclei in the promycelium, the lowermost nucleus is separated off by a septum (fig. 9)



FIGS. 1–10.—Fig. 1, pycnium, ×400. Fig. 2, cell fusion in telial primordium, ×1800. Fig. 3, teliospore initials, ×800. Fig. 4, young teliospore, ×800. Fig. 5, mature teliospore, ×800. Figs. 6, 7, stages in formation of fusion nucleus, ×1800. Fig. 8, germinating teliospore with linear row of four nuclei, ×800. Fig. 9, separation of lower promycelial cell, ×800. Fig. 10, development of infection branch, ×800.

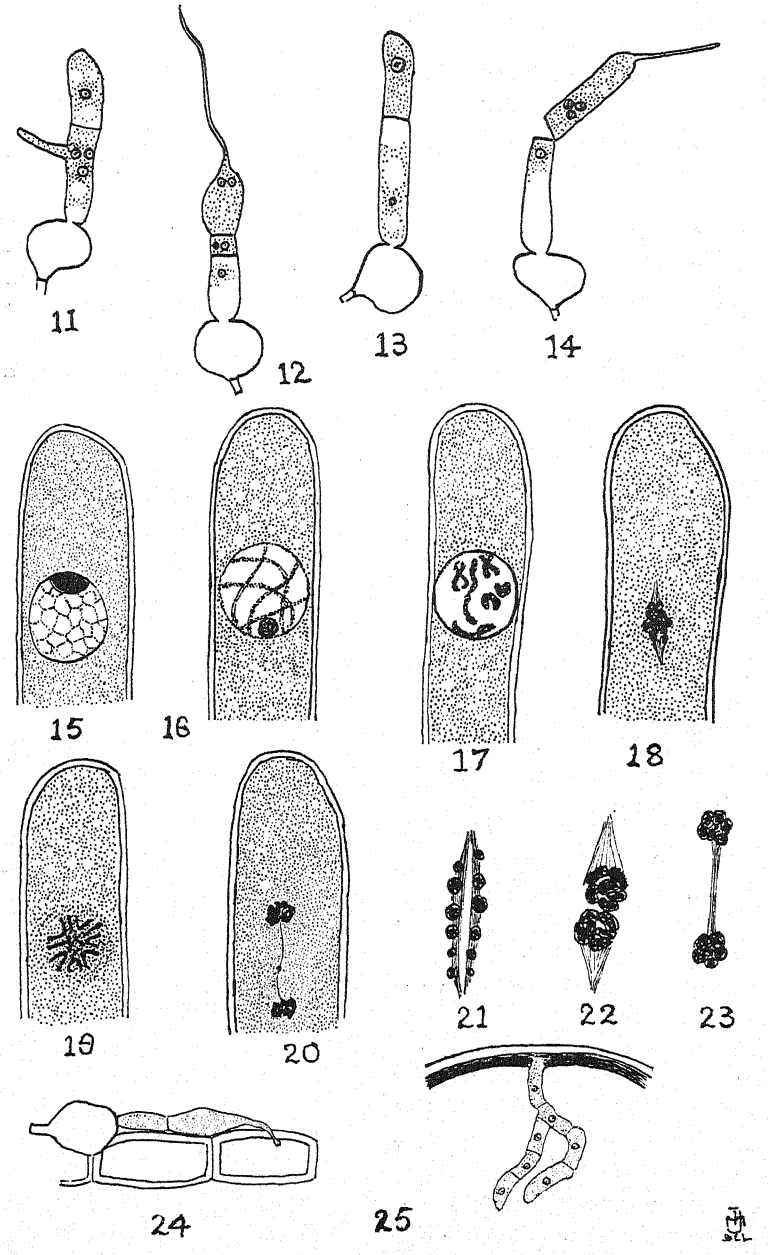
which appears to be laid out by cleavage furrows. This is facilitated by the differentiation of small vacuoles which coalesce with one another. The two-celled promycelium thus produced has an upper three-nucleate cell and a lower uninucleate cell. The apical portion of the upper cell very soon elongates into a whiplike process into which the three nuclei migrate along with the cytoplasmic contents. This whiplike structure, although usually terminal, often develops in a lateral position (fig. 10). In the lower cell the nucleus remains inactive and in later stages degenerates. The cell contents become highly vacuolated and in some instances undergo shrinkage.

In one of the observed variations the entire process of development usually found in the upper cell of the promycelium is restricted to the lower cell (fig. 11); i.e., the upper cell is uninucleate and sterile, whereas the lower one is three-nucleate and develops the infection hypha. In another instance, after the linear row of four nuclei was organized in the promycelium, two septa were laid separating three cells. The upper cell was binucleate. The middle cell showed some activity in having a single nuclear division and becoming binucleate (fig. 12), but very soon all the nuclei within the two lower cells degenerated, leaving only the upper fertile cell. In such cases the promycelium appears to be three-celled, but only one of the cells is fertile. The three-celled promycelia observed by AJREKAR and TONAPY might be of similar nature. In still another type of variation, after the first meiotic division and the separation of the two daughter nuclei, the lower one degenerates and the upper one, by further division, forms a binucleate promycelial cell (fig. 13).

The promycelium in *U. aloës* is thus usually two-celled, with one fertile cell and one sterile cell, or three-celled, owing to the presence of two sterile cells. The fertile cell develops a whiplike structure which, during infection, directly penetrates into the host and is therefore of the nature of an infection branch. In other rusts the cells of the promycelium may develop a whiplike branch in place of a sporidium when excessive moisture conditions prevail during teliospore germination. Such abnormal types of teliospore germination are common among partly submerged spores with limited oxygen supply. In *U. aloës*, however, even in a seemingly suitable environment, the development of basidiospores has never been observed. It seems certain that they are not formed. The promycelial cell has assumed the function of the sporidium and develops the infection branch. The rounding-up of promycelial cells (fig. 14) which behave like sporidia has been recorded in species of the genera *Chrysocelis* Diet. and *Acervulopsora* Thirumalachar. AJREKAR and TONAPY compared it with the genus *Barclayella* Diet. (=*Chrysomyxa*), in which, however, the rounding-up of promycelial cells has been ascribed to abnormal conditions prevailing during germination.

MEIOSIS

The following cytological details were observed for the meiotic divisions of the fusion nucleus in the promycelium. The fusion nucleus shows a finely staining reticulum of chromatin with a nucleolus which, in most of the cases observed, is situated on the side of the nucleus toward the tip of the promycelium (fig. 15). The nucleolus stains deeply with gentian-violet and haematoxylin. The first indication of the beginning of the prophase is the condensation of the chromatin into long, narrow, sinuous strands. These



FIGS. 11–25.—Figs. 11–13, variation in type of teliospore germination, ×400. Fig. 14, upper promycelial cell separating, ×400. Figs. 15–23, meiosis. Fig. 15, resting nucleus, ×1800. Fig. 16, zygonema stage in promycelium, ×1800. Fig. 17, diakinesis, ×1800. Fig. 18, first metaphase, ×1800. Fig. 19, stage before organization of spindle, ×1800. Fig. 20, anaphase, ×1800. Fig. 21, late metaphase, ×3600. Fig. 22, clumping of chromosomes at either side of equatorial plate, ×3600. Fig. 23, anaphase, ×3600. Fig. 24, penetration of infection hypha through epidermis, ×400. Fig. 25, septate mycelium composed of uninucleate cells after infection, ×800.

are distributed within the nucleus without any definite arrangement and constitute the leptotene stage. Many nuclei have been observed in the zygonema stage showing the pairing of chromosomes (fig. 16). Very soon after this pairing, the chromosomes contract abruptly in length and become short and stumpy. Six pairs of chromosomes have been counted in most of the preparations showing diakinesis (fig. 17). The nuclear membrane disappears at this stage, and the first indication of the organization of the spindle is observed. The nucleolus disappears completely just at the stage of diakinesis. Such a feature has been reported by several workers (**19, 20, 25**). OLIVE (**21**), on the other hand, working on the nuclear phenomena of *Coleosporium helianthi*, noticed the persistence of the nucleoli during all the stages of the first meiotic division. It was also stated that they undergo splitting like the chromosomes and are translocated into the two daughter nuclei. Since these observations by the several investigators have been made on very carefully fixed and stained preparations, it is probable that the behavior of the nucleolus during nuclear division varies in different species of rusts.

The early stages in organization of the spindle are obscure. Its formation usually coincides with the disappearance of the nuclear membrane in late prophase. After its organization, the translocation of the chromosomes along the spindle to the respective poles can be made out (figs. 18, 19, 21, 22). Chromosome counts made during both diakinesis and the metaphase indicate that the haploid number is six. Chromosome numbers are also known for some other rusts; about eight in *C. helianthi* (**21**), eight for *Cronartium ribicola* (**8**), six to ten in *Coleosporium sonchi-arvensis* (**14**), and five in *Puccinia malvacearum* (**2**) (inaccurately described as two by many previous investigators). The presence of a centrosome with the characteristic polar radiations and other amphiastral figures has not been noticed. SAVILE also failed to find them in any of the rust species investigated by him. Centrosomes with polar radiations have been figured by COLLEY for *Cronartium ribicola* and by OLIVE for *Coleosporium helianthi*. In the latter rust OLIVE attributed to them the function of contributing toward the formation of the cell plate. The presence or absence of the centrosomes and polar radiations might be characteristic features of the particular rust species.

Toward the later part of the anaphase the chromosomes show tendencies to become clumped (figs. 20, 23) at the poles. In many cases they were massed on either side of the equatorial region of the spindle itself (fig. 22). The telophase is soon recognized, the nucleoli and the nuclear membrane reappearing. In a short time the second meiotic division is initiated, resulting in the formation of four free nuclei. The spindles in this second division are much smaller than in the first, with the chromosomes being too minute to permit definite counting.

INOCULATION EXPERIMENTS

AJREKAR and TONAPY by a series of inoculation studies proved the autoecious nature of *U. aloës*. They further stated that the promycelial cell with its threadlike outgrowth might sooner or later become separated from the empty part of the promycelium and actually function as a basidiospore. In the present studies, germinating teliospores were placed on young leaves of *A. vera*, and the inoculated plants were inclosed in moist chambers for 48 hours, after which they were placed in a greenhouse for

observation. The inoculated portions of the leaves were fixed at different intervals (24, 48, and 72 hours after removal from the moist chambers), and microtome sections were cut to observe the various stages of penetration into the host. The whiplike germ tube, upon contact with the epidermis of the host, first forms an appressorium and enters the epidermal cell by the formation of a slender process (fig. 24). It is probable that all three nuclei enter into the host along the infection hypha, but no definite observations have been made to this effect. The three nuclei have not been recognized individually because of the fact that soon after entry they divide, and a septate mycelium is developed. This is composed entirely of uninucleate cells (fig. 25). The mycelium ramifies in the intercellular spaces, and later on the hyphae are grouped together to form the pycnial initials.

The formation of sporidia is completely suppressed irrespective of the conditions under which germinations are carried out. It is, therefore, difficult to think of monosporidial infections in the usual sense of the term. However, as each teliospore on germination has been observed to produce only one fertile promycelial cell which is equivalent with the sporidia, single teliospore infections would achieve the same end as monosporidial infections. For this purpose, single teliospores were easily picked out by adopting the dry-needle method of HANNA, and they were used for inoculating healthy leaves of *A. vera*. Successful infections were observed in ten cases, indicated by the appearance of tiny yellow specks. These gradually expanded into patches, and the development of pycnia, followed by telia, took place after 14–20 days. If these single teliospore inoculations could constitute monosporidial infection—which cannot be claimed with certainty—then it might be presumed that *U. aloës* behaves like a homothallic rust.[1] The cytological details observed during teliospore germination indicate that the nuclear cycle is not of the usual type found in *Puccinia malvacearum* but is somewhat complicated.

Discussion

In rusts and in many of the higher basidiomycetes a species is considered to be homothallic when the entire life-cycle is completed starting from a single uninucleate basidiospore. Numerous microcyclic rusts have been studied experimentally (4) and their homothallic or heterothallic nature confirmed. In *P. malvacearum* (3) the basidiospore is uninucleate and on infection develops a uninucleate mycelium. Binucleate cells appear at various points in the initiation of the telial stage, and the two nuclei which associate in a young teliospore are the products through successive mitoses of the original sporidium. According to JACKSON (15), homothallic rusts were derived from heterothallic ancestors through simplification of the life-cycle. Two kinds of homothallism are distinguished (6): those that are haplomonoecious like *P. malvacearum*, where the life-history starts with a single uninucleate sporidium, and those that are sexually heterokaryotic or miktohaplontic (16, 17). In the miktohaplontic species, the ascospore or the basidio-

[1] In a recent paper JACKSON (Trans. Roy. Soc. Canada, Sec. V, Ser. III, 38:1–32. 1944) stated that the two haploid thalli developed from + and − basidiospores are not of different sex but must be interpreted as bisexual (sexually homothallic) hermaphroditic, intrasterile, and cross-fertile. If *Puccinia graminis* is then characterized as being homothallic, according to this interpretation, DODGE (Mycologia 37:784–791. 1945) pointed out that miktohaplontic forms like *Neurospora tetrasperma* would be doubly homothallic.

spore initially is bisexual, containing the nuclei of both sexes. Among the ascomycetes *Neurospora tetrasperma* (9), *Pleurage anserina* (12), and *Gelasinospora tetrasperma* (13) are miktohaplontic. In two species of basidiomycetes—*Puccinia arenariae* (18), and *Cintractia montagnei* (23)—the promycelium remains two-celled; the two basidiospores receive two nuclei each and thus are considered bisporous. These and other basidially bisporous forms of Hymenomycetes like *Coprinus ephemerus* and *Galera tenera* (24) were grouped under miktohaplontic species by BULLER (6). Considering the life-cycle of *U. aloës*, it is evident that the basidium remains mostly two-celled, the upper cell containing three or rarely two nuclei derived after the meiotic division in the promycelium. It is probable that this is a case similar to that of *Cintractia montagnei*, *Puccinia arenariae*, and others which are considered by BULLER as basidially bisporous.

The degeneration of one or two nuclei in the lower cell of the promycelium in *U. aloës* is rather enigmatic. Usually in rusts after the meiotic divisions in the promycelium, the four nuclei are distributed in four haploid or two diploid sporidia. The degeneration of any of the nuclei would mean a loss of certain combinations of characters. Such a phenomenon, however, has been observed in some of the basidiomycetes of which two instances are cited.

a) *Endophyllum valerinae-tuberosae* is a homothallic rust with a uninucleate mycelium. In the young teliospore two nuclei are present, but no synkaryon is formed, one of the two nuclei degenerating. The remaining nucleus migrates into the promycelium, and a mitotic division results in a two-celled promycelium. The upper cell forms a uninucleate sporidium, while the nucleus in the lower cell degenerates. Degeneration of nuclei thus occurs twice within the course of the life-cycle.

b) In *Psalliota campestris* the basidium is bisporous, each spore possessing two nuclei. BUHR (5) observed that in some basidia only a single nucleus enters each basidiospore, the other two nuclei degenerating within the basidium-body. The degeneration of one or two nuclei in the lower cell of the promycelium of *U. aloës* is comparable with the condition present in the two above-mentioned cases and might indicate an aberrant mode of the sexual process.

Since the discovery of the functions of pycnia by CRAIGIE, rust species with well-developed pycnia are usually considered heterothallic (4), and in the homothallic species the pycnia are either absent or underdeveloped. In *U. aloës* the pycnial development is incomplete in the sense that no ostiole with flexuous hyphae are produced. The pycniospores are extruded out of the narrow pore through the epidermis and collect in a droplet of secreted nectar. In some of the pycnia the entire cavity is filled by pycniosporophores without any spore development. According to JACKSON (15), "the presence of spermagonia or spermatia, however, should not be considered as indisputable evidence that the species is heterothallic. It is quite possible that in species spermatia are formed but are no longer functional." JACKSON cited as evidence the instances of *Endophyllum euphorbiae-sylvaticae* and *Gymnoconia nitens*—the form with a four-celled promycelium studied by DODGE and GAISER (10)—in which the nuclei do not fuse prior to the formation of the basidium, thus suggesting a homothallic development, and yet they show development of pycnia. *E. cen-*

tranthi-rubri and one of the uninucleate strains of *G. nitens*, which possess the haplophase throughout their life-cycles, could only be homothallic. However, pycnial development has been observed, and these must be nonfunctional. In *Cystopsora oleae* the mycelia produced as a result of sporidial or aeciospore inoculations of young leaves are first multinucleate and later on dikaryotically diploid, and yet well-developed pycnia with ostiole and paraphyses as in heterothallic rusts are present (28).

JACKSON (15), in comparing the life-cycle of *Herpobasidium filicinum* with the microcyclic rusts, enumerated six distinct types of variations present in the endo- and micro-forms. The life-cycles of long-cycled rusts are mostly uniform in all essential features. The life-cycle of *U. aloës* shows that it cannot be equated with any of the types enumerated by JACKSON and thus adds another variation.

Summary

1. *Uromyces aloës* (Cke.) Magn. is a microcyclic rust developing pycnia and telia on the leaves of *Aloe vera*. The pycnia are subepidermal and remain abortive and underdeveloped in many cases.

2. The telia are subepidermal, and the teliospores germinate readily, producing usually two-celled, and rarely three-celled, promycelia. The terminal cell, which may thus be bi- or trinucleate, is fertile, while the nuclei in the lower cell, or cells, degenerate. The terminal fertile cell produces an infection hypha which penetrates into the host. Development of basidiospores has not been observed, but the terminal cell of the promycelium assumes the function of basidiospores.

3. A detailed cytological study of teliospore germination has been made. The haploid chromosome number, as determined during the meiotic divisions of the fusion nucleus in the promycelium, is six. In its type of teliospore germination, *U. aloës* has been shown to resemble *Puccinia arenariae*, *Cintractia montagnei*, and others which are considered to be basidially bisporous by BULLER.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. AJREKAR, S. L., and TONAPY, B. R. A note on the life-history of *Uromyces Aloës* (Cke.) Magn. Jour. Ind. Bot. Soc. 3:267–269. 1923.
2. ALLEN, R. F. A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum*. Phytopath. 23:572–586. 1933.
3. ASHWORTH, DOROTHY. *Puccinia malvacearum* in monosporidial culture. Trans. Brit. Mycol. Soc. 16:177–202. 1931.
4. BROWN, A. M. The sexual behaviour of several plant rusts. Can. Jour. Res. 18:18–25. 1940.
5. BUHR, H. Untersuchungen über zweisporige Hymenomyceten. Archiv. f. Protistenkunde 77:125–151. 1932.
6. BULLER, A. H. R. The diploid cell and the diplodisation process in plants and animals with special reference to the higher fungi. I. Bot. Rev. 7:335–387. 1941.
7. CHRISTMAN, A. H. Sexual reproduction in rusts. BOT. GAZ. 39:267–275. 1905.
8. COLLEY, R. H. Parasitism, morphology, and cytology of *Cronartium ribicola*. Jour. Agr. Res. 15:619–660. 1918.
9. DODGE, B. O. Nuclear phenomena associated with heterothallism and homothallism in the ascomycete *Neurospora*. Jour. Agr. Res. 35: 269–305. 1927.
10. DODGE, B. O., and GAISER, L. O. The question of nuclear fusions in the blackberry rust. Jour. Agr. Res. 32:1008–1012, 1022–1023. 1926.
11. DOIDGE, E. M. A preliminary study of the South African rusts. Part I. Bothalia 2:1–228. 1926.
12. DOWDING, E. S. The sexuality of the normal, giant, and dwarf spores of *Pleurage anserina* (Ces.) Kunze. Ann. Bot. 45:1–15. 1931.
13. ———. *Gelasinospora*, a new genus of pyrenomycetes with pitted spores. Can. Jour. Res. 9:295–305. 1933.

14. HOLDEN, R. J., and HARPER, R. A. Nuclear divisions and nuclear fusion in *Coleosporium sonchi-arvensis* Lév. Trans. Wis. Acad. Sci. 14:63–82. 1903.
15. JACKSON, H. S. The nuclear cycle in *Herpobasidium filicinum* with a discussion on the significance of homothallism in basidiomycetes. Mycologia 27:553–572. 1935.
16. KNIEP, H. Die sexualitat der niederen Pflanzen. Jena. 1928.
17. ———. Verebungserscheinungen bei Pilzen. Bibliogr. Genet. 5:371–478. 1929. (Cited from 6.)
18. LINDFORS, T. Studien über den Entwicklungsverlauf bei einigen Rostpilzen aus zytologischen und anatomischen Gesichtpunkten. Svensk. Bot. Tidskr. 18:34–37. 1924.
19. MOREAU, MME F. Les phénomènes de la sexualité chez le Uredine. Le Botaniste 13. 1914. (Original not seen.)
20. OLIVE, E. W. Sexual cell fusions and vegetative nuclear divisions in the rusts. Ann. Bot. 22:331–360. 1908.
21. OLIVE, LINDSAY S. Nuclear phenomena involved at meiosis in *Coleosporium Helianthi*. Jour. Elisha Mitchell Sci. Soc. 58:43–51. 1942.
22. ———. Morphology, cytology, and parasitism of *Thekopsora Hydrangeae*. Jour. Elisha Mitchell Sci. Soc. 59:65–67. 1943.
23. RAWITSCHER, F. Beiträge zur Kenntnis der Ustilagineen II. Zeitschr. Bot. 14:274–295. 1922.
24. SASS, J. E. A cytological basis for homothallism and heterothallism in the Agaricaceae. Amer. Jour. Bot. 16:663–701. 1929.
25. SAVILE, D. B. O. Nuclear structure and behavior in species of Uredinales. Amer. Jour. Bot. 26:585–609. 1939.
26. THIRUMALACHAR, M. J. A method for germinating and staining teleutospores. Jour. Ind. Bot. Soc. 19:71–75. 1940.
27. ———. *Phragmotelium mysorensis*, a new rust on Indian raspberry. Proc. Ind. Acad. Sci. 15:186–193. 1942.
28. ———. Development of spore-forms and the nuclear cycle in the autoecious opsis rust, *Cystopsora oleae*. BOT. GAZ. 107:74–86. 1945.

CHARACTERISTICS OF THE NITROGEN-FIXING ENZYME SYSTEM IN NOSTOC MUSCORUM[1]

R. H. BURRIS AND P. W. WILSON

Introduction

Fixation of molecular nitrogen by the blue-green alga *Nostoc muscorum* is of special interest, since in some respects this system represents an intermediate between fixation by the free-living heterotrophic bacterium, *Azotobacter*, and the symbiotic combination of *Rhizobium* plus leguminous plant. Previous studies from this and other laboratories (2, 4, 9), dealing with the properties of the enzyme systems responsible for nitrogen fixation by *Azotobacter* and by inoculated red clover plants (*Trifolium pratense* with *R. trifolii*), established that these two systems resemble one another in many critical respects—dissociation constant (K_{N_2}), response to H_2 and to CO. Extension of such investigations to *Nostoc* was recognized but delayed because of lack of suitable technique. Fixation by this organism is slow, and replicates often vary considerably. As studies on the nitrogen-fixing system are as yet limited to growing cultures, these defects in experimental method discouraged enzyme studies; it appeared unlikely that sufficient precision would be obtained for investigations of this nature. We have overcome to a considerable extent this handicap by use of many-fold replication and by application of the isotopic tracer method. Although the data still lack the precision achieved with either *Azotobacter* or in-

[1] This research was supported in part by grants from the Rockefeller Foundation and the Wisconsin Alumni Research Foundation.

oculated red clover plants, they are sufficiently precise for comparison with these two.

Methods

The pure culture of *N. muscorum* employed was kindly furnished by Dr. F. E. ALLISON. Inoculum for the experiments was prepared: 25-ml. portions of approximately 2-week-old cultures in Roux bottles were transferred to 100 ml. of fresh ALLISON's nitrogen-free medium with 1% sucrose (1) in 1-liter Roux bottles, and the cultures were incubated about 1 week in a south window at a room temperature of approximately 25° C. Six of these 125-ml. cultures were then combined with 800 ml. of fresh medium and shaken vigorously with glass beads in a 2-liter flask. Sterile air was passed through the flask rapidly to keep the algal clumps suspended, while 35-ml. portions were pipetted into 1-liter flat-bottomed pyrex bottles. Combined-nitrogen compounds were added to individual bottles as sterile neutral solutions in 1 ml. volume. The bottles were stoppered in pairs, and gases were removed and added through cotton plugs to the two bottles simultaneously. Unless otherwise noted, the pO_2 was initially 0.2 atm., and the pCO_2, 0.05 atm., in all bottles. The other gases were varied as indicated by particular experiments. The culture bottles were incubated in a greenhouse at a normal temperature of 22°–25° C.; during the late spring and summer months the temperatures exceeded 25°, and it was necessary to shade the bottles from direct sunlight. The bottles were illuminated at night by 200-watt bulbs in reflectors at a distance of 30 inches. Cultures were usually analyzed after incubation for 4–7 days. The cells from flasks to which combined nitrogen was added were washed by centrifugation before Kjeldahl analysis.

Experimentation

THE pN_2 FUNCTION

The partial pressure of nitrogen (pN_2) was varied from 0.02 to 0.75 atm.; helium was added to atmospheric pressure, correction being made for the 2.5% N_2 in the helium sample. Each treatment was replicated four times in the nitrogen-fixation series and twice in the combined-nitrogen series (300 p.p.m. NH_4^+–N). Variations arising from lack of uniformity in inoculum, in alterations of growth rate through disturbing the algal pad, and from differences in light should be partially eliminated by averaging of the replicates. Data from four trials are summarized in table 1. Considerable variation in response still exists both within and among the experiments even when the averages are used. In general, the data agree with those obtained in similar experiments made with *Azotobacter* (10) and with red clover plants (9), but two quantitative differences are suggested.

First, there is some evidence that assimilation of fixed nitrogen is not entirely independent of the pN_2. If true, it indicates that the level of NH_4^+–N used was insufficient to inhibit completely nitrogen fixation, a result quite different from that noted with the other agents (11). Because of its implication for the mechanism of fixation, this suggestion is worth keeping in mind, though the evidence is certainly not conclusive, since the differences are neither marked nor consistent in the sense that uptake of NH_4^+–N always decreased when the pN_2 was lowered.

Second, the dissociation constant (K_{N_2}) appears to be at least 0.10 atm. (see data in last column of table 1; value of the pN_2 at which fixation is one-half maximum). With *Azotobacter* (10) the

value is 0.01–0.02 atm. and with red clover, about 0.05 atm. (9). If the data are plotted for estimation of K_{N_2} ($1/pN_2$ against $1/v$) decision must be made as to the best measure of v, the velocity constant of fixation. As insufficient data are available to decide this definitely, calculations were made of two possibilities: (*a*) a linear rate, i.e., the final total nitrogen fixed was used as a measure of v; (*b*) a logarithmic rate in which the first-order velocity constant, $k = 2.303/\text{time}$ in

TABLE 1*

ASSIMILATION OF N_2 AND NH_4^+–N AS FUNCTION OF THE pN_2 BY NOSTOC MUSCORUM

pN_2 (ATM.)	NH_4^+–N ASSIMILATED (MG./35 ML.)				N_2 FIXED (MG./35 ML.)				
	Experiment†			Av.‡	Experiment†				Av.‡
	1	2	3		1	2	3	4	
0.02	3.95	.....	5.98	95	0.57	0.61	0.55	.....	28
.05	3.88	.....	5.20	84	0.93	0.79	0.89	0.54	43
.10	.....	6.14	4.57	77	0.81	1.00	1.08	0.48	47
.20	3.38	7.11	6.23	90	1.22	1.28	1.04	0.74	57
.40	4.02	7.01	5.29	89	1.16	2.16	2.03	0.78	85
0.75	4.41	7.87	6.02	100	1.81	2.67	1.85	1.63	100

* Values in table corrected for nitrogen at start. Blank indicates not included or sample lost.

†

Expt.	Time	Mg. N at start
1	7 days	0.51
2	4 days	.44
3	5 days	.45
4	4 days	0.25

‡ Relative to value at 0.75 atm. = 100.

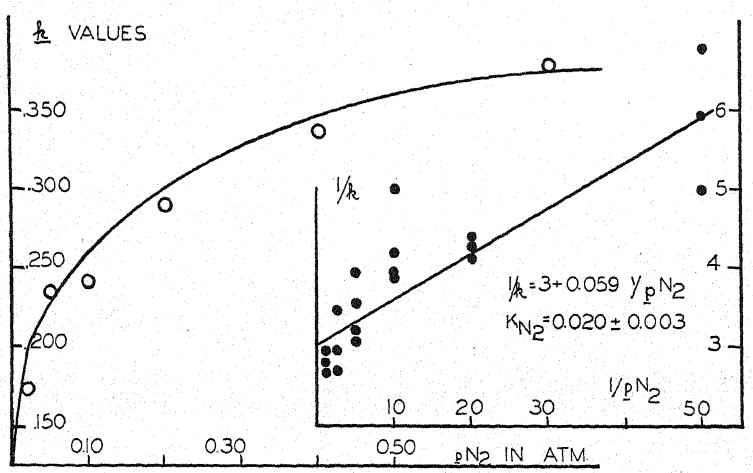


FIG. 1.—Response of *Nostoc muscorum* to partial pressure of nitrogen in atmosphere. Values of k in each experiment adjusted to a common k_{max} for the combined plotting. Each point is average of three to four separate determinations, each of which is based on four replicates.

Insert: Calculation of K_{N_2} from these data. Individual determinations shown.

days×log (final N/ initial N), was used. The following values for the K_{N_2} were obtained under the first assumption: 0.028, 0.042, 0.041, and 0.058 atm. Combining the data from all experiments led to a value of 0.032 atm., but the appropriate statistical test (6) indicated that a linear function did not properly describe the combined data, so this value is suspect.

The data based on an assumed logarithmic rate are shown in figure 1. In this case the combined data showed less evidence of nonlinearity, but a more desirable fit to a straight line is still needed; the F value (6) was just at the 5% level of significance. Also, several of the observed rates at the higher partial pressures of N_2 (0.40 and 0.75 atm.) are greater than the estimated k_{max}. Although the excesses are not more than can be accounted for by experimental error, their existence casts doubt on the accuracy of the estimated K_{N_2}. For these reasons the indicated value, 0.02 ± 0.003 atm., is highly provisional, and its close agreement with that observed with *Azotobacter* (10) is likely only fortuitous.

Experiments concerned with influence of CO on the fixation process included four in which partial pressures of N_2 of 0.75 and 0.10 atm. were compared in the absence of the inhibitor. The average value of k for the former was 0.414 and for the latter, 0.268. Calculation of K_{N_2} from the formula (10)

$$K_{N_2} = \frac{k - k'}{k'/p' - k/p}$$

for these four trials led to a value of 0.07 ± 0.019 atm. (cf. with red clover equal to 0.05 atm.). Although a more precise evaluation of K_{N_2} for the *Nostoc* system awaits additional experiments in which the rate of reaction is determined by periodic analyses, present estimates justify the conclusion that this significant enzyme constant does not differ markedly from those of the nitrogen-fixing systems in *Azotobacter* and *Trifolium*.

TABLE 2

EFFECT OF H_2 ON UPTAKE OF FREE AND COMBINED NITROGEN BY NOSTOC MUSCORUM (ISOTOPIC EXPERIMENTS)

Expt.	Time in hours	pN_2 (atm.)	pO_2 (atm.)	Atom % excess N^{15}	
				Vacuum	0.6 atm. H_2
5	70	0.16	0.24	0.267	0.172
				0.224	0.179
				0.313	0.182
6	144	.20	.20	5.44	5.20
7	36	.20	.20	0.171	0.050
8*	28	0.20	0.20	6.43	6.74
				6.60	6.46
				6.82	6.61

* 50 p.p.m. NH_4^+–N (32% atom excess N^{15}) added in this experiment.

THE pH_2 FUNCTION

A critical test for comparison of nitrogen-fixation systems is their response to molecular hydrogen. Although this gas is inert for most enzyme systems other than those involving hydrogenase, it *specifically* and *competitively* inhibits nitrogen fixation by both *Azotobacter*

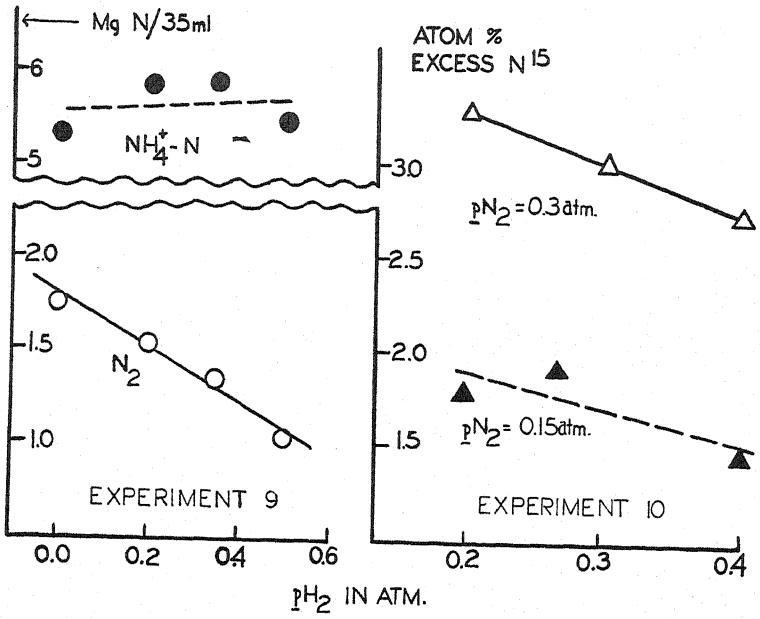


FIG. 2.—Effect of hydrogen gas on uptake of free and combined nitrogen by *Nostoc muscorum*. *Left*, comparison of assimilation of N_2 and NH_4^+–N (total-nitrogen experiment, $pN_2 = 0.2$, $pO_2 = 0.175$ atm., helium to fill). *Right*, effect of pN_2 on action of H_2 (isotopic experiment, $pO_2 = 0.3$ atm., vacuum).

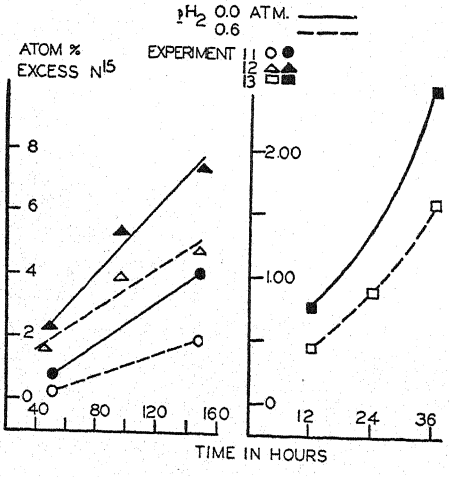


FIG. 3.—Effect of hydrogen on rate of nitrogen fixation by *Nostoc muscorum*.

(13) and red clover (9). Because of the importance of the effect of hydrogen, a more sensitive method was sought for verification of data obtained in the total-nitrogen type of experiment. It was found that many of the irregularities could be eliminated if isotopic N^{15} were used to measure fixation as has been previously described (3). The absolute quantity of cells initially added as inoculum is not especially critical in governing the amount of N^{15} they assimilate, and the experiments can be completed in a relatively short time, minimizing confusing secondary reactions. The chief limitation of the method at present results from the lack of sufficient equipment to handle numerous replicates in a single experiment. For this reason we have not used it in the pN_2 experiments, although the procedure is certainly recommended whenever facilities become available.

For test of the method *Azotobacter* cultures were grown in the presence and absence of H_2 (0.6 atm.); the excess of N^{15} found in the cultures after 28 hours over that normally present was: without

H_2, 3.52, 3.44, and 3.44; with H_2, 2.66, 3.06, and 2.67 atom %. In a second experiment after 19 hours the values obtained were: without H_2, 1.45, 1.43, and 1.66; with H_2, 0.96, 1.07, and 0.70 atom %.

Data in table 2 and in figures 2 and 3 demonstrate that H_2 likewise specifically inhibits nitrogen fixation by *N. muscorum*. This is evidenced by the results obtained with N^{15} (table 2, figs. 2, 3) as well as by those with the more traditional total-nitrogen method (fig. 2, expt. 9). The specificity of the effect is illustrated by the data of experiments 8 (table 2) and 9 (fig. 2), which indicate that the effect of H_2 is confined to assimilation of N_2 and has little, if any, effect on uptake of NH_4^+–N. Whether the effect is competitive or not cannot be stated without further additional study, but the results of experiment 10 are consistent with the view of competition. The results of experiments 11, 12, and 13 (fig. 3) suggest that, as previously noted with the other nitrogen-fixing agents, the effect is on the rate of fixation. From these results with H_2 it is concluded that the nitrogen-fixing system in *Nostoc* is closely related to that in *Azotobacter* and in red clover.

The *p*CO function

Further evidence of the similarity of the nitrogen-fixation system in *Nostoc* with that in *Azotobacter* and in red clover is furnished by experiments dealing with the effect of CO. This gas does not possess the specificity for inhibition of fixation shown by H_2, since it may affect the assimilation of nitrate and, at relatively high concentrations, of ammonium (12). Nevertheless, because of quantitative differences in the effect on fixation of N_2 as compared with assimilation of the combined forms, its use as an inhibitor provides another valuable criterion for establishing similarities and differences among biological nitrogen-fixing systems.

TABLE 3

Effect of CO on fixation of N_2 by Nostoc muscorum (isotopic experiments)

Expt.	Time in hours	*p*CO (atm.)	Atom % excess N^{15}
^{14} pO_2=0.2 atm. pN_2=0.2	38	0.00	0.108
			.131
			.138
		.006	.039
			.033
			.035
^{15} pO_2=0.2 atm. pN_2=0.2	28	.000	.115
			.144
		.0005	.107
		.002	.060
		0.004	0.009

TABLE 4

Action of CO on the assimilation of ammonium nitrogen by Nostoc muscorum (isotopic experiment 16)

Atmosphere	Atom % N^{15} excess
Air	8.17
Air + *p*CO 0.0005*	8.26
Air + " 0.001	8.41
Air + " 0.002	8.52
Air + " 0.004	8.09
Air + " 0.006	8.42
Air + " 0.02	8.33
0.80 vac., 0.004 CO, 0.20 O_2	7.58
0.80 H_2, 0.004 CO, 0.20 O_2	7.95
0.80 He, 0.004 CO, 0.20 O_2	7.78

* All values in this column expressed as atm.

The response of *N. muscorum* to CO was determined in isotopic and total-nitrogen experiments; the results are summarized in tables 3–6 and in figure 4. The specificity of the action of CO at a *p*CO of 0.006 atm. (0.6%) or less is

demonstrated by comparison of the data in tables 3 and 4. The ammonium ion in experiment 16 (table 4) was supplied as 50 p.p.m. $(NH_4)_2SO_4$ containing 32% atom excess N^{15}. After 24 hours' incubation the cells were recovered by centrifugation, washed, and analyzed for N^{15}. Since the level of the heavy isotope was approximately the same in all samples, it is concluded that CO at the concentrations employed has no effect on assimilation of NH_4^+ and is therefore specific for fixation of N_2. Similar results were obtained in experiments 17–22 (tables 5, 6) which were based on total-nitrogen data and which included both nitrate and ammonium nitrogen. A rate experiment is shown in the insert of figure 4.

TABLE 5

EFFECT OF CO ON ASSIMILATION OF FREE NITROGEN (TOTAL-NITROGEN EXPERIMENTS)

pCO (ATM.)	EXPERIMENT 17 (96 HR.)*		EXPERIMENT 18 (96 HR.)*	
	N_2 fixed/35 ml. (mg.)	Relative	N_2 fixed/35 ml. (mg.)	Relative
0.000.....	2.09	100	1.11	100
.00025....	2.67	127	1.17	105
.0005.....	1.54	74	1.06	90
.00075....	1.83	87	1.06	90
.0010.....	1.84	88	0.89	80
.0015.....	0.89	33	0.34	31
.002.....	0.19	9	0.13	12
0.003.....	0.05	2	0.05	5

* $pN_2 = 0.75$, $pO_2 = 0.2$ atm. in both experiments.

In general, the response of *Nostoc* to CO is similar to that already observed in *Azotobacter* (7, 12) and red clover (8). The effective levels are of the same order of magnitude, and the inhibition at these low pCO values appears to be restricted to fixation of N_2. The differences between the influence of CO on uptake of NO_3^- and NH_4^+ established in respirometer experiments with *Azotobacter* (12) are not too much in evidence (table 6), but, in view of the experimental error and the relatively long time required for the total-nitrogen experiments, the expected comparatively small quantitative difference could be readily hidden. More important differences are suggested by the data dealing with the nature of the inhibition shown in figure 4, in which the average values from four experiments with a pN_2 of 0.10 atm. and from

TABLE 6

EFFECT OF CO ON UPTAKE OF COMBINED NITROGEN BY NOSTOC MUSCORUM (TOTAL-NITROGEN EXPERIMENTS)

EXPT.*	pCO (ATM.)	300 P.P.M. NO_3^--N		300 P.P.M. NH_4^+-N	
		Uptake/35 ml. (mg.)	Relative	Uptake/35 ml. (mg.)	Relative
19 (120 hr.).....	0.000	3.29	100	6.07	100
	0.003	2.49	76	5.05	83
20 (96 hr.).....	0.000	2.32	100	4.65	100
	0.003	1.86	80	4.49	96
21 (96 hr.).....	0.000	2.80	100	5.35	100
	0.002	3.24	115	4.73	88
22 (96 hr.).....	0.000	2.81	100	3.71	100
	0.002	2.68	95	3.40	92

* $pN_2 = 0.75$, $pO_2 = 0.20$ atm. in all experiments.

five experiments with a pN_2 of 0.75 atm. are plotted. Each point at the lower pN_2 represents eight replicates and, at the higher, fourteen replicates. Two results are noteworthy: (*a*) the quantity of nitrogen fixed at a pCO of 0.0005 atm. and a pN_2 of 0.75 atm. was consistently greater than that in the absence of CO and (*b*) competitive inhibition is indicated, since CO exerted a greater percentage inhibition at the lower pN_2. A

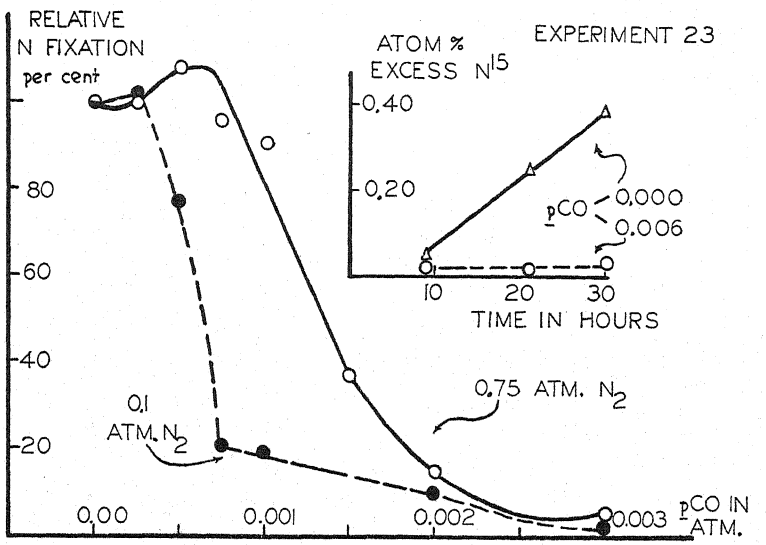


FIG. 4.—Effect of CO on fixation of nitrogen by *Nostoc muscorum* at different pN_2. (expts. 24–28). *Insert:* Time course of effect of CO measured by fixation of N^{15}.

stimulative action of CO has not been observed with either *Azotobacter* or red clover, and the inhibition of *Azotobacter*, at least, is largely, if not entirely, noncompetitive (5).

Summary

1. The properties of the nitrogen-fixing enzyme system in *Nostoc muscorum* appear to be very similar to those already noted in the free-living bacterium, *Azotobacter* and in the symbiotic system of *Trifolium pratense* and *Rhizobium trifolii*. The half-maximum rate of fixation by *Nostoc* (Michaelis dissociation constant, K_{N_2}), although not determined with great precision, apparently does not differ markedly from those observed for *Azotobacter* (0.02 atm.) and red clover (0.05 atm.).

2. The response to H_2 and CO is much the same as with the *Azotobacter* and the symbiotic system. The data indicate considerable unity in the properties of the nitrogen-fixation systems in the three types of organisms. The specific activity of CO in very low concentrations and of H_2 are unusual phenomena; this adds significance to their action on the enzyme systems from different sources.

3. Although the evidence strongly suggests that nitrogen fixation is mediated by similar enzyme systems in *Azotobacter*, *Trifolium*, and *Nostoc*, one point of difference may exist which might be significant. In contrast to *Azotobacter* (and possibly red clover), CO is more effective as an inhibitor of N_2 fixation by *Nostoc* at a pN_2 of 0.10 atm.

than at a pN_2 of 0.75; this is suggestive of competitive inhibition. The half-maximum rate of fixation occurs at approximately a pCO of 0.0006 (pN_2 of 0.10 atm.) and at a pCO of 0.0014 (pN_2 of 0.75 atm.). These values are intermediate between those noted with *Azotobacter* (0.004–0.005 atm.) and with red clover (0.0002 atm.).

DEPARTMENT OF AGRICULTURAL BACTERIOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ALLISON, F. E.; HOOVER, S. R.; and MORRIS, H. J. Physiological studies with the nitrogen-fixing alga, *Nostoc muscorum*. BOT. GAZ. **98**: 433–463. 1937.
2. BURK, D., and BURRIS, R. H. Biochemical nitrogen fixation. Ann. Rev. Biochem. **10**:587–618. 1941.
3. BURRIS, R. H.; EPPLING, F. J.; WAHLIN, H. B.; and WILSON, P. W. Detection of nitrogen fixation with isotopic nitrogen. Jour. Biol. Chem. **148**:349–357. 1943.
4. BURRIS, R. H., and WILSON, P. W. Biological nitrogen fixation. Ann. Rev. Biochem. **14**:685–708. 1945.
5. EBERSOLE, E. R.; GUTTENTAG, C.; and WILSON, P. W. Nature of carbon monoxide inhibition of biological nitrogen fixation. Arch. Biochem. **3**: 399–418. 1944.
6. EISENHART, C., and WILSON, P. W. Statistical methods and control in bacteriology. Bact. Rev. **7**:57–137. 1943.
7. LIND, C. J., and WILSON, P. W. Carbon monoxide inhibition of nitrogen fixation by *Azotobacter*. Arch. Biochem. **1**:59–72. 1942.
8. ———, and ———. Mechanism of biological nitrogen fixation. VIII. Carbon monoxide as an inhibitor for nitrogen fixation by red clover. Jour. Amer. Chem. Soc. **63**:3511–3514. 1941.
9. WILSON, P. W. The biochemistry of symbiotic nitrogen fixation. University of Wisconsin Press, Madison, Wis. 1940.
10. WILSON, P. W.; BURRIS, R. H.; and LIND, C. J. The dissociation constant in nitrogen fixation by *Azotobacter*. Proc. Nat. Acad. Sci. (U.S.) **28**:243–250. 1942.
11. WILSON, P. W.; HULL, J. F.; and BURRIS, R. H. Competition between free and combined nitrogen in nutrition of *Azotobacter*. Proc. Nat. Acad. Sci. (U.S.) **29**:289–294. 1943.
12. WILSON, P. W., and LIND, C. J. Carbon monoxide inhibition of *Azotobacter* in microrespiration experiments. Jour. Bact. **45**:219–232. 1943.
13. WYSS, O., and WILSON, P. W. Mechanism of biological nitrogen fixation. VI. Inhibition of *Azotobacter* by hydrogen. Proc. Nat. Acad. Sci. (U.S.) **27**:162–168. 1941.

CYTOLOGICAL STUDIES IN THE ALISMACEAE

WALTER V. BROWN

Introduction

The present study was undertaken to determine whether cytology can be of aid to a clearer understanding of the taxonomically difficult genus *Sagittaria* and to determine with more certainty the chromosome numbers and morphology of species in this genus and *Alisma*.

OLESON (9) reviewed the literature concerning chromosome numbers in this family through 1941. Her count for *Alisma plantago*, $2n=14$, is at variance with those of LIEHR (4), $2n=12$, and of WULFF (13), $2n=10$. HAGERUP (2) reported this species to have $n=7$ and *A. lanceolatum* $n=14$, thus confirming OLESON's determination. In *Sagittaria*, also, a range in numbers has been reported. LIEHR counted $2n=16$ for *S. sagittifolia*, TAYLOR (11) $2n=ca.$ 20 for *S. montevidensis*, NAWA (8) $2n=20$ for *S. trifolia* var. *sinensis*, LOHAMMAR (5) $2n=22$ for *S. sagittifolia* and *S. natans*, MORINAGA and FUKUSHIMA (6) $2n=22$ for *S. trifolia*

var. *sinensis*, and SHINKE (10) $2n=22$ for *S. aginashi*. OLESON found two more species to have $2n=22$—*S. latifolia* and *S. rigida*. All these workers studied sections of root tips and stated that they encountered considerable difficulty in counting the long and twisted chromosomes.

Material and methods

Mitotic chromosomes were studied in all species, smears being made of the root tip in aceto-carmine. Inasmuch as it was desired to spread the chromosomes a great deal, very little tissue was used on each slide, and considerable pressure was exerted on the cover slip to break the cell and, to a limited extent, to scatter the chromosomes. Absolute-acetic was used as the fixative (7 parts absolute alcohol to 3 parts glacial acetic acid) for a short time only, one-half hour to an hour. The material was smeared immediately after and directly from the fixative. All plants studied were collected by the author, except *S. arifolia* and *A. subcordatum*, from Colorado Springs, Colorado, furnished by Mr. ROBERT LIVINGSTON, and *S. sagittifolia*, which was purchased. Potted plants were grown in an artificial pool in the greenhouse at Duke University for ready access to material and then grown to maturity for herbarium specimens, which are in the author's personal herbarium.

Observations and discussion

ALISMA

The chromosome numbers of all genera and species of Alismaceae so far studied cytologically are listed in table 1. The author's counts of $2n=14$ (fig. 6) for *A. triviale* and $2n=28$ (fig. 4) for *A. subcordatum* substantiate the counts of OLESON and HAGERUP. It is probably true, as OLESON stated, that the basic number in this genus is seven. The author disagrees, however, with OLESON's findings on the morphology of the chromosomes of *Alisma*. She stated that "they may be classed as long, medium and short." This is true, but, as figures 1, 2, 4, and 6 show, these categories are arbitrary and practically useless. The author finds a fairly regular series of chromosomes decreasing in length from about 22 to 8 μ. OLESON further stated: "Four chromosomes are long, 2 having median and 2 subterminal constrictions; 6 of these are of medium length and 4 are short." This is quite different from the author's findings. Whereas OLESON reported two long chromosomes with median constrictions, the author finds that ten chromosomes have the constriction so located (fig. 6). OLESON's four short chromosomes are the only ones with subterminal constrictions in the author's material.

FERNALD (1) recently revised the taxonomic concept of American species of *Alisma*. He finds that American *Alisma* is distinct from the European *A. plantago-aquatica* L. There are two species of *Alisma* in the United States, *A. triviale* Pursh, the more northern species with larger flowering parts, and *A. subcordatum* Raf., more southern with smaller flowering parts. OLESON's *A. plantago* is probably *A. triviale*, the diploid species, as found in the present investigation. *A. subcordatum* is tetraploid. HAGERUP reported the European species *A. lanceolatum* to be tetraploid with $n=14$. Thus, there is one diploid and one tetraploid species in Europe and one diploid and one tetrapoloid species in North America. The polyploid *A. subcordatum* appears to have two sets of chromosomes, each of which is very simi-

lar in shape and length to the set found in *A. triviale* (cf. figs. 4 and 6 as well as figs. 1 and 2).

SAGITTARIA

In *Sagittaria*, as in *Alisma*, there has been disagreement as to both chromosome number and morphology. The reports of SHINKE, LOHAMMAR, MORINAGA and FUKUSHIMA, and OLESON agree that the various species studied by them have $2n=22$. The counts of LIEHR and NAWA have since been checked and corrected by later investigators. The count of Taylor, $2n=ca.$ 20, is indefinite, as the author himself states. The present paper reports the numbers in fifteen species of *Sagittaria* (table 1); all are the same—$2n=22$. Nineteen species have now been studied cytologically with no polyploidy or variation in chromosome number between species.

TABLE 1

CHROMOSOME NUMBERS IN ALISMACEAE

Genus and species	n	$2n$	Authority
Alisma plantago L.	.....	12	LIEHR (4)
A. plantago L.	.....	10	WULFF (13)
A. plantago L.	.....	14	OLESON (9)
A. plantago-aquatica L.	7	.....	HAGERUP (2)
A. triviale Pursh	.....	14	BROWN
A. lanceolatum With.=A. stenophyllum Asch. & Gr.	14	.....	HAGERUP (2)
A. subcordatum Raf.	.....	28	BROWN
Echinodorus ranunculoides L.	9	.....	HAGERUP (2)
Limnophyton obtusifolium (L.) Miq.	12	.....	NARASIMHA MURTHY (7)
Sagittaria montevidensis Cham.+Schl.	*ca.* 20	.....	TAYLOR (11)
S. stagnorum Small	.....	22	BROWN
S. kurziana Gluck	.....	22	BROWN
S. kurziana X stagnorum	.....	22	BROWN
S. subulata var. gracillima J. G. Smith	.....	22	BROWN
S. natans Pallas	.....	22	LOHAMMAR (5)
S. teres Wats.	.....	22	BROWN
S. graminea Michx.	.....	22	BROWN
S. falcata Pursh	.....	22	BROWN
S. lancifolia L.	.....	22	BROWN
S. rigida Pursh	.....	22	OLESON (9)
S. rigida Pursh	.....	22	BROWN
S. weatherbiana	.....	22	BROWN
S. arifolia Nutt.	.....	22	BROWN
S. latifolia Willd.	11	22	OLESON (9)
S. latifolia Willd.	.....	22	BROWN
S. pubescens Muhl.	.....	22	BROWN
S. longirostra (Micheli) J. G. Smith	.....	22	BROWN
S. engelmanniana J. G. Smith	.....	22	BROWN
S. sagittifolia L.	.....	16	LIEHR (4)
S. sagittifolia L.	.....	22	LOHAMMAR (5)
S. sagittifolia L.	.....	22	BROWN
S. trifolia var. sinensis (Sims) Makino	.....	20	NAWA (8)
S. trifolia var. sinensis (Sims) Makino	.....	22	MORINAGA and FUKUSHIMA (6)
S. aginashi Makino	11	22	SHINKE (10)

All species appear to have the same type of genom. TAYLOR, MORINAGA and FUKUSHIMA, and SHINKE agree that there is one pair of long chromosomes with median constriction. OLESON found two pairs with median constrictions, as did LOHAMMAR. SHINKE's description of the chromosome complement of *S.*



FIGS. 1–9.—Figs. 1–3, idiograms of: fig. 1, *Alisma triviale;* fig. 2, *A. subcordatum;* fig. 3, *Sagittaria.* Figs. 4–9, chromosome complements of: fig. 4, *A. subcordatum;* fig. 5, *S. lorata;* fig. 6, *A. triviale;* fig. 7, *S. graminea;* fig. 8, *S. latifolia;* fig. 9, *S. falcata.* All chromosome drawings reproduced at ×800.

aginashi—two very long chromosomes having central fiber-attachment constrictions, eighteen rod shaped with subterminal constrictions, and two very short ones rod shaped but fiber-attachment point not designated—is the most nearly correct description in the literature. Add that the very small chromosome has a submedian constriction, and he has described the chromosome set as found in all species of *Sagittaria* yet studied cytologically. Figure 3 represents a typical or average idiogram for *Sagittaria* based on measurements of the complements of ten species and on one or more sets for each species. The study was not set up to determine and measure chromosome length with great accuracy, nor were sufficient chromosome sets of each species measured. There may be significant differences in individual chromosome lengths between species, but this study did not determine that. This average idiogram consists of one very long chromosome about 22 μ long with a central primary constriction, one very short chromosome about 6.5 μ long with submedian constriction, and nine chromosomes with subterminal constrictions and varying in length: 16, 14.5, 13, 12.5, 11.5, 11, 10.5, 10, and 9 μ, respectively. The short chromosome in the complement has a submedian constriction dividing it into two arms, one of which is twice as long as the other. The shorter arm is approximately twice as long as the short arm in the chromosomes with subterminal constrictions. This chromosome is definitely not in the latter group, however, as all chromosomes with subterminal constrictions have short arms that appear almost spherical, with a diameter slightly less than the diameter of the long arm. This is not true of the short arm of this shortest chromosome.

The idiograms of *Alisma* and *Sagittaria* are quite different (cf. figs. 1 and 3). On the basis of LEWITSKY's (3) hypothesis—that primitive members of a group have mostly isobrachial chromosomes, while derived members have mostly heterobrachial—it would appear that *Alisma* is a rather primitive genus while *Sagittaria* is more advanced or derived. If four of the five isobrachial chromosomes of *Alisma* divided to form eight heterobrachial units, a complement similar in number to that found in *Sagittaria* could be formed. Similarly, a genus phylogenetically intermediate between these two would be expected to have an intermediate number of chromosomes and from two to four isobrachial members. HAGERUP (2) reported *Echinodorus ranunculoides* to have $n=9$, but the morphology of these chromosomes was not determined. It might be expected, however, to have three long isobrachial chromosomes and six with subterminal constrictions.

Summary

1. The chromosome numbers of two species of *Alisma* and fifteen species of *Sagittaria* are reported.

2. The basic number in *Alisma* is $n=7$, consisting of five isobrachial and two heterobrachial chromosomes. *A. triviale* is diploid, *A. subcordatum* is tetraploid.

3. All nineteen species of *Sagittaria* that have been studied cytologically have $2n=22$, consisting of one pair of long chromosomes with median constriction, one pair of very short chromosomes with submedian constriction, and nine pairs of chromosomes with subterminal constrictions.

4. It is probable that *Alisma* is a primitive genus, whereas *Sagittaria* is a more advanced or derived type.

Acknowledgment is due to Dr. GEORGE L. CHURCH of Brown University, Dr. H. L. BLOMQUIST and Dr. LEWIS E. ANDERSON of Duke University, and Dr. KARL SAX of Harvard University for assistance and valuable suggestions.

BIOLOGICAL LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

1. FERNALD, M. L. North American representatives of *Alisma plantago-aquatica*. Rhodora **48**: 86–87. 1946.
2. HAGERUP, O. Notes on some boreal polyploids. Hereditas **30**:152–160. 1944.
3. LEWITSKY, G. A. The karyotype in systematics. Bull. Appl. Bot., Genet. and Plant Breeding **27**: 187–240. 1931.
4. LIEHR, O. Ist die angenommene Verwandtschaft der Helobiae und Polycarpiae auch in ihrer Cytologie zu erkennen? Cohns Beitr. Biol. Pflanz. **13**:135–220. 1916.
5. LOHAMMAR, G. The chromosome numbers of *Sagittaria natans* Pallas and *S. sagittifolia* L. Svensk. Bot. Tidskr. **25**:32–35. 1931.
6. MORINAGA, T., and FUKUSHIMA, E. Chromosome numbers in cultivated plants. III. Bot. Mag. Tokyo **45**:140–145. 1931.
7. NARASIMHA MURTHY, S. K. Half-yearly Jour. Mysore Univ. **7**:1. 1933. (Cited by TISCHLER [12].)
8. NAWA, N. Some cytological observations in *Tricyrtis*, *Sagittaria* and *Lilium*. Bot. Mag. Tokyo **42**:33–36. 1928.
9. OLESON, E. M. Chromosomes of some Alismaceae. BOT. GAZ. **103**:198–202. 1941.
10. SHINKE, N. Spiral structure of chromosomes in meiosis in *Sagittaria aginashi*. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. **9**:366–392. 1934.
11. TAYLOR, W. R. Chromosome constrictions as distinguishing characteristics in plants. Amer. Jour. Bot. **12**:238–244. 1925.
12. TISCHLER, G. Pflanzliche Chromosomen-Zahlen. Tab. Biol. **16**:162–218. 1938.
13. WULFF, H. D. Chromosomenstudien an der Schleswig-Holsteinischen angiospermen Flora. III. Ber. Deut. Bot. Ges. **57**:84–91. 1939.

THE ROLE OF ORGANIC MATTER, ESPECIALLY MANURE, IN THE NUTRITION OF RICE[1]

JAMES BONNER

Introduction

During the course of an investigation of the effects of manure on the growth of plants, it was found that rice responded to the addition of this material by striking increases in growth. The effect of manure was first noted in sand cultures supplied with a standard inorganic nutrient and could not be duplicated by alteration of the composition of the nutrient solution within wide limits. Neither was the effect of the manure on growth of rice owing to any possible effects on structure or tilth of the substrate, since rice is grown with the roots completely submerged, so that the effects of organic matter on soil structure and hence on the aeration of the soil, which are so important with other plants, are of little significance here. The data to be discussed below show, on the contrary, that rice cultures, under the conditions obtaining in these experiments, are able to utilize organic nitrogen and that manure influences the growth of rice through its content of organic nitrogenous compounds.

[1] Report of work supported by the John Ellis Fund. The author is indebted to Mr. ELLIS and to Dr. A. D. SHAMEL for their continued interest and many helpful suggestions during the course of this work.

Material and methods

The rice used was of the variety Colusa. It was germinated in a warm greenhouse in washed river sand contained in flats, and the seedlings were used when the plants had attained a height of 15–20 cm. to the top of the tallest leaf. No nutrient was added to the seedling cultures. Experiments were set up either in 1-gallon crocks, 1½-quart cans, or in 20-cc. shell vials, the size of container depending on the purpose of the experiment and the length of time which the plants were to be grown. With the plants in crocks or cans, sand was used as a substrate. The containers were without bottom drainage and were filled with nutrient solution so that the roots were completely submerged. The nutrient was drained out and replaced by fresh at intervals of 1–14 days in the various experiments. Plants could be grown to maturity in the 1-gallon crocks and could be grown for 30–60 days in the 1½-quart cans. Many of the experiments were, however, done with seedling plants grown over a period of only 2 weeks, for reasons which will become apparent below. In these experiments the rice seedlings were transplanted singly to 20-cc. shell vials containing 15 cc. of nutrient solution and stoppered with corks. Each plant was held in place in a slit in the side of a cork, and each cork was further provided with a small central hole for the addition of water. These vials were contained in lots of twenty in boxes so constructed that the vials and hence the roots of the seedling were kept in darkness within the box. Plants so cultured were grown in the air-conditioned greenhouse for 2 weeks at a constant temperature of 80° F. Growth under these conditions was in general excellent, the fresh weight of plants approximately doubling during the 2-week period.

TABLE 1

REPRODUCIBILITY OF RICE PLANTS GROWN INDIVIDUALLY IN 20-CC. SHELL VIALS AND HARVESTED IN LOTS OF TEN. EXPERIMENT M-59. INITIAL WEIGHT OF PLANTS, 1.73 GM.

Row no.	Fresh weight (gm./10 pl.)	Row no.	Fresh weight (gm./10 pl.)
1	4.61	10	3.83
2	4.30	11	4.09
3	3.74	12	3.62
4	3.95	13	4.10
5	4.26	14	3.98
6	4.27	15	4.59
7	3.71	16	4.20
8	3.50	17	4.62
9	4.74	18	4.15
Av.	4.12	Av.	4.13

The degree of variability between lots of rice seedlings grown in vials is shown in table 1. Each figure represents the fresh weight at harvest of ten rice seedlings grown for 2 weeks as outlined above. The weights differ from the overall mean by an average of 7% of the mean.

The manure used in this investigation was a well-rotted, air-dry, ground, commercial steer product. The nutrient solutions used were based on those described by HOAGLAND and ARNON (9) and by VICKERY *et al* (18). The HOAGLAND solution supplies all the nitrogen as nitrate, while the VICKERY solution supplies all the nitrogen as ammonium (table 2). In both cases the nutrient was diluted to one-half of the standard concentration, since other work, not here described, has established that young rice plants have only a relatively low tolerance to salts. The initial pH of the nitrate nutrient was 5.5, of the ammonium nutrient 6.2.

Results

PRELIMINARY INVESTIGATIONS.—It is generally known that ammonium salts

are more readily utilized than nitrates by young rice plants as a source of nitrogen. This has been noted by KELLNER (11), NAGAOKA (13), KELLEY (10), ESPINO (7), TRELEASE and PAULINO (17), THELIN and BEAUMONT (16), and PARDO (14), among others. KELLEY has shown (10) that in rice-paddy soils nitrate formation is slow and that nitrogen tends to accumulate as ammonia. Preliminary experiments confirmed the preferential utilization of ammonium nitrogen by rice. Plants supplied with nitrate in the standard nutrient grew poorly and showed typical symptoms of nitrogen deficiency while those supplied with ammonium nitrogen grew vigorously and showed only slight symptoms of nitrogen deficiency. Incorporation of solid manure in the sand of sand cultures resulted in increased growth of plants either in nitrate- or in ammonium-containing nutrient solution (table 3). The manure was mixed in the sand with which the containers were filled, and the nutrient was drained out and renewed once per week. Ten per cent by volume of manure mixed in the sand gave approximately the optimum effect in increasing growth, and this effect was reflected in height, number of tillers, and weight of plants produced. Similar results were obtained in two further experiments with nitrate nutrient. In another series of experiments, the addition of manure to ammonium nutrient cultures resulted in better growth than could be achieved by variation within wide limits of the major elements in composition of the nutrient solution. For this purpose in one experiment use was made of a triangular design in which ammonium, potassium, and calcium were varied with respect to one an-

TABLE 2

COMPOSITIONS OF NITRATE- AND AMMONIUM-CONTAINING NUTRIENT SOLUTIONS USED

SALT*	MOLARITY IN NUTRIENT	
	Nitrate-containing nutrient	Ammonium-containing nutrient
Calcium nitrate	0.0025	.....
Calcium chloride	.....	0.0045
Magnesium sulfate	.001	.001
Potassium nitrate	.0025	.....
Ammonium sulfate	.....	.0045
Potassium dihydrogen phosphate	0.0005	0.003

* In addition, micronutrients were added to supply the following concentrations: Fe, 1 p.p.m.; B, 0.5 p.p.m.; Mn, 0.5 p.p.m.; Zn, 0.1 p.p.m.; Cu, 0.2 p.p.m.; Mo, 0.1 p.p.m.

TABLE 3

EFFECT OF MANURE ON GROWTH OF RICE IN SAND CULTURE. ONE-HALF STRENGTH HOAGLAND'S SOLUTION AS NUTRIENT. EIGHTEEN PLANTS PER TREATMENT. RANDOMIZED PLOT DESIGN. EXPERIMENT M-1. INITIAL HEIGHT OF PLANTS APPROXIMATELY 10 CM.

	HEIGHT OF PLANT IN CM. AFTER			NO. OF TILLERS AFTER 39 DAYS	DRY WEIGHT (GM./PL.) AFTER 39 DAYS
	11 days	25 days	39 days		
Control: nutrient only	10.4	13.5	17.0	1.3	2.49
5% manure in sand by volume	15.9	19.6	21.9	3.7	7.32
10% manure in sand by volume	18.4	22.6	26.8	5.9	16.92
25% manure in sand by volume	14.3	20.9	27.7	4.4	9.53

other while the anions sulphate, chloride, and phosphate were maintained at constant concentrations. Table 4 gives the compositions of the three stock solutions employed; these three solutions were combined by ninths to obtain nineteen different nutrient solutions. Rice seedlings were transplanted from flats to 1-gallon crocks, two plants per crock, and arranged in randomized design with four

TABLE 4

COMPOSITION OF THREE STOCK SOLUTIONS USED IN MAKING UP A NUTRIENT TRIANGLE FOR VARIATION OF AMMONIUM, CALCIUM, AND POTASSIUM IONS

Salt	Stock solution A* (ammonium stock millimols/liter)	Stock solution B* (potassium stock millimols/liter)	Stock solution C* (calcium stock millimols/liter)
$(NH_4)_2SO_4$	3	.....	.....
$NH_4H_2PO_4$	2	.....	.....
NH_4Cl	6	.....	.....
$MgSO_4$	1	.....	.....
K_2SO_4	.....	3	.....
KH_2PO_4	.....	2	.....
KCl	.....	6	.....
$MgSO_4$	.....	1	.....
$CaSO_4$	.....	.....	3.0
$Ca(H_2PO_4)_2$	.....	.....	1.0
$CaCl_2$	.....	.....	1.5
$MgSO_4$	.....	.....	1.0

* Each stock solution additionally received minor elements in the concentrations given in table 1.

plots. Each of the nineteen nutrient solutions was tested on eight crocks (sixteen plants). Growth took place in the greenhouse, and the nutrient was renewed every 1–2 weeks. In addition to the several nutrient solutions, a twentieth treatment was included in which manure was incorporated in the sand (10% by volume) and to which solution no. 9 (equal volumes of the three stock solutions) was added. Growth was allowed to continue until the grains were filled and mature. Because the experiment was started in late summer, flowering and seed setting took place while the plants were still relatively small. The plants were harvested, dried, and weighed. Results are given in figure 1. It is apparent that the high nitrogen treatments allowed greater growth of plants than treatments which supplied less nitrogen. However, the addition of manure allowed more vigorous growth than any other treatment in both total dry weight and yield of grain. This effect of added manure was manifest within 1 week of the inception of the experiment.

The experiments of table 3 and figure 1, confirmed in detail in four further experiments with rice in sand culture, establish an influence of manure on the growth of rice. The nature of this influence is the subject of the following sections.

GROWTH OF RICE SEEDLINGS.—In order to study the nature of the factors in manure which promote the growth of rice, use was made of rice seedlings grown in 20-cc. shell vials as described above. The influence of pH of nutrient was investigated in an experiment in which ammonium nutrient was buffered at various pH levels with phosphate buffer. Acidity of the nutrient appeared to be without great effect over the range of pH 4.5–7.0 (table 5). Similarly, growth of rice under these conditions did not appear to be limited by the concentration of available iron, since increasing the iron concentration by four times increased growth by less than 10%. Similar investigation of each of the micronutrients showed that no increase in concentration of any of them significantly affected growth under the conditions of the experiments.

Table 6 gives the results of an experiment in which additional ammonium was added (as ammonium sulfate) to the nutrient solution. Only small increases

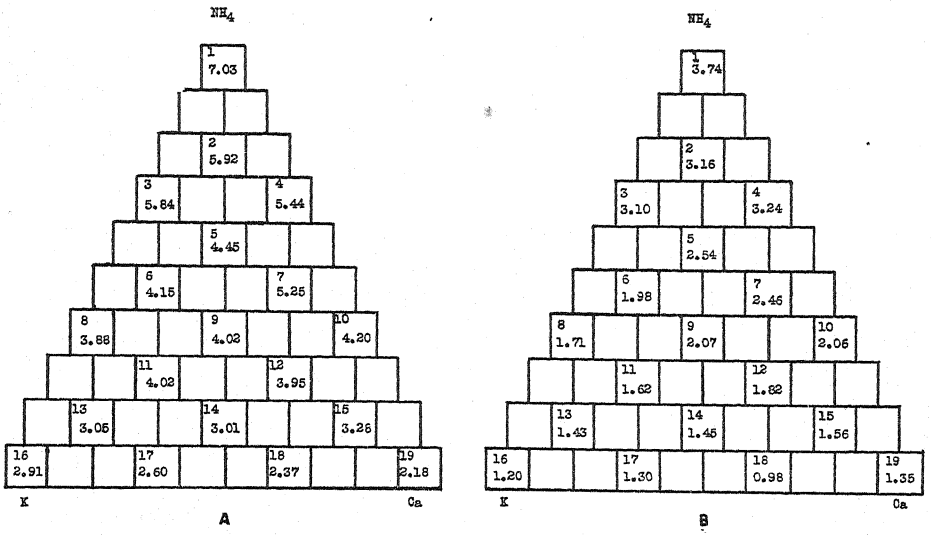


FIG. 1.—Triangular experiment on effect of nutrition on growth and seed production of rice plants in sand culture: *A*, dry weight in gm./2 pl. *B*, seeds in gm./2 pl. Solution no. 9 plus manure: dry wt., 16.86 gm./2 pl.; seed wt., 8.98 gm./2 pl.

in growth resulted from increasing the concentration of ammonium in the medium. An increase of ammonium by more than about 25% above the concentration in the basal medium resulted in the appearance of toxicity symptoms. Nitrate-nitrogen appeared to be incapable of supporting the growth of rice seedlings under the present conditions (table 11).

ACTIVITY AND FRACTIONATION OF MANURE.—Addition of manure to rice grown in vials with ammonium nutrient resulted in increased growth (table 7). Addition of 200 mg. dry weight of screened manure per rice plant increased growth, as measured by fresh weight, by 38% over the control plants after a period of 2 weeks. The manure was therefore subjected to fractionation by various procedures, one of which was exhaustive extraction with acetone in a Soxhlet extractor. Table 7 shows that the solid manure residue left after extraction is even more effective than the original manure, since an aliquot of the residue which represented 200 mg. of initial manure caused an increase in fresh weight above the controls of 106%. The material extracted by acetone, on the other hand, was found to be toxic. It would appear, therefore, that acetone

TABLE 5

EFFECT OF VARYING PH ON GROWTH OF RICE SEEDLINGS IN VIALS. 0.025 M PHOSPHATE BUFFER IN AMMONIUM NUTRIENT. EXPERIMENT M-16

pH OF NUTRIENT	No. OF PLANTS	FRESH WEIGHT IN GM./10 PL.		
		Stems	Roots	Total
4.5	20	1.14	1.45	2.59
5.0	20	1.29	1.51	2.80
5.5	20	1.08	1.15	2.23
6.0	20	1.09	1.22	2.31
6.5	20	1.25	1.55	2.80
7.0	20	1.10	1.07	2.17
7.5	20	0.99	0.95	1.94
8.0	20	0.94	0.80	1.74

removes some toxic material from the manure, resulting in an enhancement of the growth-promoting activity of the residual acetone-insoluble material.

Only a small portion of the growth-promoting activity of manure is soluble in water, and, on the contrary, manure retained its growth-promoting activity after exhaustive water extraction. Experiments with rice in sand culture rather than in vials confirmed this water insolubility of the principle which promotes the growth of rice. The growth-promoting factor would appear to be mainly associated with the solid portion of the manure.

INFLUENCE OF KNOWN SUBSTANCES ON RICE.—The fact that water extracts of manure are ineffective in promoting growth of rice indicates that the active principle of manure cannot be a vitamin of the B group such as has been found to influence the growth of certain plants in some cases (3, 4, 5). Table 8 shows that thiamin, pyridoxin, nicotinic acid, pantothenic acid, and biotin were all without influence on the growth of rice. Yeast extract, on the contrary, was ex-

TABLE 6

EFFECT OF VARIOUS CONCENTRATIONS OF AMMONIUM SULFATE ON GROWTH OF RICE SEEDLINGS IN VIALS IN AMMONIUM NUTRIENT. EXPERIMENT M-37

ADDENDUM	AMOUNT OF N AS NH_4 (MG./PL.)			FRESH WEIGHT IN GM./10 PLANTS	
	In basal nutrient	Added	Total	Total	Growth
Initial (approx.)	.....	.....	.....	1.86	.....
Final controls	1.80	0.00	1.80	3.72	1.86
+ 0.2 mg. $(NH_4)_2SO_4$	1.80	0.046	1.85	3.65	1.79
+ 0.6 mg. $(NH_4)_2SO_4$	1.80	0.14	1.94	4.16	2.30
+ 2.0 mg. $(NH_4)_2SO_4$	1.80	0.46	2.26	3.60	1.74
+ 6.0 mg. $(NH_4)_2SO_4$ (M-45)	1.80	1.40	3.20	Toxic	.....
+20.0 mg. $(NH_4)_2SO_4$	1.80	4.60	6.40	Toxic	.....

TABLE 7

EFFECT OF VARIOUS FRACTIONS OF MANURE ON GROWTH OF RICE SEEDLINGS IN VIALS. EXPERIMENT M-11. AMMONIUM NUTRIENT

TREATMENT	AMOUNT OF MANURE REPRESENTED (MG.)	NO. OF PLANTS	FRESH WEIGHT IN GM./10 PLANTS			
			Tops	Roots	Total	Growth
Initial plants (approx.)	.....	.....	.....	.....	2.23	.....
Control	.....	70	2.67	1.90	4.57	2.34
Initial manure	200	10	2.60	2.85	5.45	3.22
Water-extracted manure	200	10	2.70	2.73	5.43	3.20
Acetone-extracted manure	200	10	3.46	3.58	7.04	4.81
Acetone extract	200	10	1.66	1.12	2.78	Toxic
Water extract of acetone-extracted manure	200	10	2.74	2.39	5.13	2.90

ceedingly effective in increasing growth. Various other known chemical compounds were tried in numerous experiments. All nonnitrogenous substances tested were found to be ineffective (table 9): Nitrogen-containing compounds, on the contrary, were effective in increasing growth of seedling rice plants, as shown in the summarized data of table 10. Three amino acids and asparagin were effective, while peptone and egg albumin were particularly active. That it is actually the nitrogen of nitrogenous organic compounds which is of importance in the response of rice plants is indicated by the experiment of table 11. In this experiment rice plants were grown in nutrient solution containing nitrate as the source of nitrogen. In the basal nutrient essentially no growth took place during the 2-week experimental period. Addition of further nitrate to the medium was without effect. Addition of ammonium sulfate, of yeast extract, or of egg albumin resulted in substantial growth. Thus, the nitrogen of organic compounds, even of egg albumin, is apparently available to rice plants under the present experimental conditions. Similar results were obtained with seedlings grown in a nitrogen-free medium.[2] Under these conditions, manure or egg albumin was able to serve as the sole source of nitrogen for the growth of rice plants (table 12).

TABLE 8

LACK OF EFFECT OF VARIOUS GROWTH SUBSTANCES ON GROWTH OF RICE SEEDLINGS IN VIALS. AMMONIUM NUTRIENT. EXPERIMENT M-22

ADDENDUM	No. OF PLANTS	FRESH WEIGHT IN GM./10 PLANTS		
		Tops	Roots	Total
None (control).....	40	2.38	2.53	4.91
B_1, 10γ/vial.....	20	2.36	2.76	5.12
B_6, 10γ/vial.....	20	2.42	2.45	4.87
Nicotinic acid, 10γ/vial.....	20	2.40	2.52	4.92
Pantothenic acid, 10γ/vial.....	20	2.20	2.74	4.94
Biotin, 0.01γ/vial....	20	2.41	2.37	4.78
Yeast extract, 40 mg./vial.....	20	4.48	4.99	9.47

TABLE 9

INFLUENCE OF VARIOUS NITROGEN-FREE COMPOUNDS ON GROWTH OF SEEDLING RICE PLANTS IN VIALS. AMMONIUM NUTRIENT. SUMMARY OF VARIOUS EXPERIMENTS

SUBSTANCE	No. OF EXPTS. IN WHICH TESTED	CONCENTRATIONS		FINAL FRESH WEIGHT AT HIGHEST NONLETHAL CONC. (% OF CONTROL)
		Range tested (mg./vial)	Highest non-lethal conc. (mg./vial)	
Glucose.....	1	10	10	92
Sucrose.....	2	10–40	10	50
Starch.....	1	10	10	92
Malic acid...	3	1–10	10	91
Pyruvic acid.	1	10	10	102
Malonic acid.	2	3–10	3	74
Fumaric acid.	3	3–10	10	102
Citric acid...	5	1–10	5	102
Succinic acid.	5	1–10	5	99

TABLE 10

INFLUENCE OF VARIOUS NITROGEN-CONTAINING COMPOUNDS ON GROWTH OF RICE SEEDLINGS IN AMMONIUM NUTRIENT. SUMMARY OF VARIOUS EXPERIMENTS

Substance	Expt. no.	Concentration used (mg./vial)	Growth in fresh weight (% of control)
Asparagin.....	M-22	10	195
Aspartic acid.....	M-32	3	131
Glycine.....	M-29	10	152
Glutamic acid....	M-31	10	200
Peptone.....	M-39	20	232
Egg albumin.....	M-39	20	271

[2] The nitrogen-free nutrient used in this and subsequent experiments contained $MgSO_4$, .001 M; KH_2PO_4, .003 M; $CaCl_2$, .002 M; minor elements and iron as shown in table 2.

Table 13 gives a quantitative comparison of the effectiveness of various sources of nitrogen in supporting the growth of rice in ammonium-containing nutrient. In the experiment of table 13, good growth was made by plants growing in the basal nutrient medium which supplied 1.8 mg. nitrogen per plant as ammonium sulfate. The addition of more nitrogen as ammonium sulfate exerted but little effect on growth, particularly since the amount of nitrogen which could be added was small owing to the toxicity of ammonium sulfate (table 6). Addition of nitrogenous organic matter in the quantities found to be beneficial to growth added significantly more nitrogen. Thus, addition of 20 mg. of egg albumin per plant added 3.3 mg. of nitrogen or approximately 1.5 times as much nitrogen as could be added as ammonium sulfate without toxicity.

The data obtained thus far can then be interpreted as follows. Rice plants grown in solution or sand culture require ammonium nitrogen. The amount which they are able to utilize over an experimental period of 1–2 weeks is greater than the amount which can be supplied without toxicity under the present conditions, owing to the high concentrations of ammonium sulfate. The nitrogen deficiency thus induced can apparently be met with other forms of reduced nitrogen which may be introduced into the medium. The nitrogen deficiency can also be overcome simply by frequent periodic renewal of the nutrient solution. Table 14 shows that if the solution was renewed daily or on alternate days, substantially more growth of rice seedlings took place than when the nutrient was renewed at less frequent intervals.

It is well known that pH of the medium is influenced by the form in which nitrogen is supplied, ammonium-containing media tending to become more

TABLE 11

EFFECT OF VARIOUS FORMS OF NITROGEN ON GROWTH OF RICE SEEDLINGS IN VIALS. BASAL NUTRIENT HOAGLAND'S DILUTED 2X, NITRATE AS NITROGEN SOURCE. EXPERIMENT M-38

ADDENDUM	NO. OF PLANTS	FRESH WEIGHT IN GM./10 PLANTS			
		Tops	Roots	Total	Growth
Initial plants	70	.....	.....	1.65	.....
Final control	40	0.95	0.77	1.72	0.07
+KNO_3, 10 mg./pl.	10	1.03	0.79	1.82	0.17
+$(NH_4)_2SO_4$, 2 mg./pl.	10	2.78	1.98	4.76	3.11
+yeast extract, 40 mg./pl.	10	3.01	2.06	5.07	3.42
+egg albumin, 20 mg./pl.	10	2.66	1.73	4.39	2.74

TABLE 12

MANURE AND EGG ALBUMIN AS SOLE SOURCES OF NITROGEN FOR GROWTH OF SEEDLING RICE PLANTS IN VIALS. NITROGEN-FREE NUTRIENT. EXPERIMENT M-56

ADDENDUM	NO. OF PLANTS	FRESH WEIGHT IN GM./10 PL.			
		Tops	Roots	Total	Growth
Initial plants	30	.....	.....	1.78	.....
Final control	50	0.78	0.74	1.52	.....
+100 mg. manure	10	1.44	1.63	3.07	1.29
+20 mg. egg albumin	10	1.81	1.43	3.24	1.46

acid as nitrogen is utilized and nitrate media tending to become more alkaline under the same circumstances. The influence of organic nitrogen on the growth of rice is not, however, exerted through effects on pH of the medium, as was shown by experiments in which the medium was buffered at pH 5.5 with phosphate buffer. Under these conditions the pH of the nutrient medium changed at most by 0.5 pH unit during the growth period of 2 weeks. Egg albumin and manure exerted their characteristic effects in increasing growth in the buffered medium.

TABLE 13

COMPARISON OF EFFECTIVENESS OF VARIOUS SOURCES OF NITROGEN IN PROMOTING GROWTH OF RICE SEEDLINGS IN VIALS. AMMONIUM NUTRIENT. BASAL MEDIUM CONTAINS APPROXIMATELY 1.8 MG. AMMONIUM NITROGEN PER PLANT. EXPERIMENT M-39

ADDENDUM	AMOUNT OF ADDENDUM	AMOUNT OF N IN ADDENDUM	FRESH WEIGHT IN GM./10 PLANTS	
			Total	Growth
Initial plants	.....	.....	2.14	.....
Final control plants	None	.....	3.67	1.53
Ammonium sulfate	2 mg.	0.46 mg.	4.03	1.89
Egg albumin	20 mg.	3.30 mg.	6.29	4.15
Peptone	20 mg.	3.30 mg.	5.69	3.55
Acetone-extracted manure (from M-32)	300 mg.	3.42 mg.	6.28	4.14

THE ACTIVE PRINCIPLE OF MANURE.—Nitrogen analyses were carried out for the various nitrogenous compounds contained in the well-rotted manure. Acetone-extracted manure contained over 1% nitrogen. Of this nitrogen, 93% was in the form of hot-water-insoluble compounds, proteins, etc., and only 7% in water-soluble form. In table 13 it was shown that addition of nitrogen to rice seedlings in the form of acetone-extracted manure exerted an effect quantitatively similar to that exerted by adding the same amount of nitrogen as egg albumin. These quantitative aspects were verified in an experiment, summarized in figure 2, in which the effects of varied concentrations of manure and egg albumin were compared. In both cases addition of 1 mg. of nitrogen per plant resulted in a substantial growth increase; addition of 3 mg. gave a larger increase, while addition of 10–11 mg. was toxic. Thus, the effects of manure and of egg albumin on growth of rice are quantitatively similar, on a nitrogen basis, even though fifteen times as much

TABLE 14

EFFECT OF VARYING FREQUENCIES OF NUTRIENT RENEWAL ON RATE OF GROWTH OF RICE SEEDLINGS IN VIALS. AMMONIUM NUTRIENT. EXPERIMENT M-58. EACH FIGURE BASED ON TWENTY PLANTS

TREATMENT	FREQUENCY OF NUTRIENT RENEWAL	FRESH WEIGHT IN GM./10 PL.	
		Total	Growth
Initial plants	.....	2.00	.....
Grown 2 weeks	Once/14 days	4.34	2.34
Grown 2 weeks	Once/7 days	4.28	2.28
Grown 2 weeks	Once/4 days	4.45	2.45
Grown 2 weeks	Once/2 days	5.97	3.97
Grown 2 weeks	Once/day	7.66	5.66

weight of manure is required to supply a given amount of nitrogen as is required in the form of egg albumin.

It has been shown that the water extract of acetone-extracted manure is scarcely effective in promoting the growth of rice and that the activity resides in the water-insoluble residue (table 7). As shown above, also, very little of the nitrogen of manure is water soluble. This fact is then in accordance with the view that the growth-promoting activity of manure for rice resides in the nitrogenous compounds of the manure.

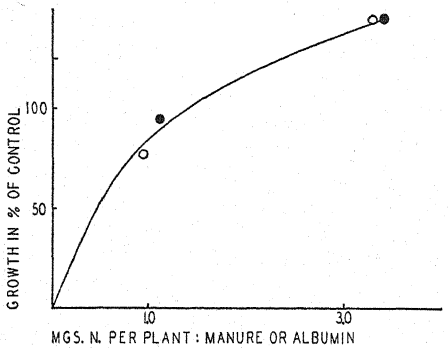


FIG. 2.—Effects of supplementary nitrogen, supplied as manure or as egg albumin, on growth of rice seedlings in vials in ammonium nutrient. Solid circles represent manure; open circles represent egg albumin.

TABLE 15

NONAVAILABILITY OF NITROGEN OF EGG ALBUMIN FOR SEEDLING RICE PLANTS GROWN IN ASEPTIC CULTURE. EXPERIMENT M-53. PLANTS GROWN IN NITROGEN-FREE BASAL NUTRIENT

Addendum	Mg./Cc.	Mgs. N per plant	No. of plants	Fresh weight after 30 days (gm./10pl.)
None.....	.....	.....	40	1.23
Ammonium sulfate.....	0.5	1.0	40	1.68
Egg albumin...	0.5	0.8	40	1.31

It would appear highly unlikely that rice plants should be able to utilize the nitrogen of protein directly. That bacteria may intervene in making protein nitrogen available for rice plants is indicated by the experiment of table 15, in which rice plants were grown under aseptic conditions in test tubes. The test tubes were approximately 1 inch in diameter by 14 inches long, and in the bottom of each 2 inches of river sand was placed. To this was added a nitrogen-free basal nutrient with or without various addenda. The whole was stoppered with cotton, autoclaved, and inoculated with aseptic rice seeds. Growth was measured as fresh weight of plants produced after 1 month. Growth in the nitrogen-free basal nutrient resulted in production of seedlings having symptoms of nitrogen deficiency. Addition of nitrogen as ammonium sulfate increased growth very considerably and relieved the symptoms of nitrogen deficiency. Egg albumin did not increase growth significantly and did not relieve the symptoms of nitrogen deficiency. Quantitatively similar results were obtained in a second experiment. It may be concluded that protein nitrogen is much less available under aseptic than under nonaseptic conditions.

Discussion

It is generally agreed that the addition of organic matter to the soil is essential to the maintenance of fertility over the years. The organic matter may be in the form of crop residues, cover crops, or farm manure; and the beneficial effects on crop yields may be determined not only in the field but in pot cultures, under experimental conditions. The nature of the effect of organic matter on soil fertility has, therefore, been the subject of intensive investigation.

Work on soils in recent years has em-

phasized the dominant role of soil structure in soil fertility. That organic matter is essential to the formation and maintenance of soil aggregates and structure has been shown by many workers and has been discussed in detail by BAVER (1), RUSSEL (15), WILLIAMS (19), MARTIN (12), and others. Whether organic matter further influences plant growth by supplying specific organic compounds which are directly utilized by the higher plant and which benefit or influence its growth in any way is debatable. It has been demonstrated beyond any doubt that many plants can grow normally in a strictly inorganic medium. It has been claimed on various occasions that addition of organic matter to inorganic nutrient media has increased the growth of one or another species of plant. In each instance that has been carefully and fully investigated, however, this claim has turned out to be only partially true, in the sense that, by alteration of the inorganic nutrient or of the environment, conditions could be found under which response of the plant to organic matter disappeared. Thus BOTTOMLEY (5) failed to provide an optimum medium for the *Lemna* with which he worked. Addition of organic matter caused increases in growth of *Lemna* under his conditions, but later workers (CLARK and ROLLER, 6) showed that even better growth could be obtained by decreasing the salt content of the medium and making provision for increased available iron. BOTTOMLEY undoubtedly supplied iron in the organic matter which he added. Recent work with *Spirodela* by GORHAM (8) has again suggested a beneficial influence of organic matter (manure), but no detailed analysis of the nature of the effect has been made. In a series of papers BONNER *et al.* (3, 4) described work which indicated that addition of certain pure organic substances increased the growth of various species of plants grown in sand culture with inorganic nutrient. These results have been confirmed only partially by the work of others. It is clear that in the original experiments special conditions of environment and nutrition prevailed, conditions under which unusually large responses to the added organic substances were obtained. In the case of *Cosmos*, which was investigated more intensively, alterations of the environmental conditions were at least as effective as the addition of organic compounds in increasing growth (BONNER, 2). Under conditions which allowed optimal growth, no effects could be obtained from added organic substances.

In the present work a systematic search was made for evidence as to whether organic matter, specifically manure, might contain special organic substances capable of causing increased growth of plants grown under the best possible environmental and nutritional conditions. Precautions were taken to rule out all effects of added manure on the physical properties of the medium. In all, sixteen species have been tested for response to manure. The tests have been carried out under a wide variety of conditions and have entailed over 120 individual experiments, each experiment in turn involving from a few to over 600 plants. Four cases were found in which the first experiment showed an apparent growth response to added manure, namely, *Poa*, *Impatiens*,[3] beet, and rice. Repetition of the experiment failed to yield a duplicate result in the case of beet, while the effect with *Impatiens* was too small for further quantitative investigation. The effect of manure on *Poa* was, however, analyzed in detail, and it was

[3] The experiments with *Poa* and *Impatiens* were carried out in co-operation with ROBERT DORLAND.

shown that *Poa*, owing to a particularly high iron requirement, suffered from iron deficiency in the conventional nutrient medium. Addition of manure merely supplied available iron, and the added growth effect of manure could be duplicated by ferric sulfate or ferric tartrate. Similarly, the effect of manure on the growth of rice has been analyzed in detail. Rice is particularly adapted to utilize ammonium rather than nitrate nitrogen, but even relatively low concentrations of ammonium are toxic to it. Several forms of organic nitrogen are available to the rice plant, including protein. Manure is shown to function in rice nutrition as a source of organic nitrogen and may serve as the sole source of nitrogen or may supplement ammonium nitrogen in the medium. Since the intervention of bacteria has been indicated to be probable in the utilization of organic nitrogen by rice, it is possible that organic forms such as protein act as reservoirs from which ammonia is slowly liberated by microbial activity.

Even in the two cases of response to manure which were found, then, the growth response on analysis has turned out to be related to the nutrition of the plant, iron nutrition in one case, nitrogen nutrition in the other. No evidence was found in the present investigation for any important role of specific organic substances contained in manure in regulating plant growth.

Summary

1. Rice plants grown in sand or solution culture were found to respond to the addition of manure with marked increases in growth. This effect of manure could not be duplicated by variations of the composition of the major or minor elements of the nutrient solution within wide limits. A systematic investigation of the nature of the effect of manure and other kinds of organic matter on the growth of rice was therefore undertaken.

2. The findings of earlier investigators that rice seedlings utilize ammonium rather than nitrate as a source of nitrogen were confirmed. In addition, it was found that ammonium nitrogen is toxic to the rice seedling at relatively low concentrations.

3. Manure and other kinds of nitrogenous organic matter are able to serve as sources of nitrogen for cultures of rice seedlings. The nitrogen of protein, peptone, and various amino acids is shown to be available to rice seedlings. These compounds are probably decomposed by micro-organisms before their nitrogen is utilized by the plant, since under aseptic conditions the nitrogen of protein does not appear to be utilized by rice.

4. The influence of manure on the growth of rice under the present experimental conditions is shown to be quantitatively attributable to the organic nitrogen content of the manure. The nitrogen of organic matter appears to serve as a supplement to the relatively low concentrations of free ammonium salts which are tolerated by the plant.

5. No evidence was found to suggest the presence in manure of a specific organic substance important in plant growth.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

LITERATURE CITED

1. BAVER, L. D. Soil physics. New York. 1940.
2. BONNER, J. Effects of application of thiamin to *Cosmos*. BOT. GAZ. 104:475–479. 1943.
3. BONNER, J., and GREENE, J. Experiments on the relation of vitamin B_1 to the growth of green plants. BOT. GAZ. 100:226–237. 1938.

4. ———, and ———. Further experiments on the relation of vitamin B_1 to the growth of green plants. BOT. GAZ. 101:491–500. 1939.
5. BOTTOMLEY, W. B. Some effects of organic growth-promoting substances on the growth of *Lemna* in mineral culture solutions. Proc. Roy. Soc. Lon. B. 89:481–508. 1917.
6. CLARK, N. A., and ROLLER, E. M. Auximones and the growth of the green plant. Soil Sci. 17: 193–199. 1924.
7. ESPINO, R. Some aspects of the salt requirements of young rice plants. Philippine Jour. Sci. 16:455–525. 1920.
8. GORHAM, P. R. Growth factor studies with *Spirodela polyrrhiza*. Thesis. Calif. Inst. of Tech. Pasadena. 1943.
9. HOAGLAND, D. R., and ARNON, D. I. The water culture method of growing plants without soil. Univ. Calif. Coll. of Agr. Circ. 347. 1938.
10. KELLEY, W. P. The assimilation of nitrogen by rice. Hawaii Sta. Bull. 24. 1911.
11. KELLNER, O. Agricultur-chemische Studien über die Reiscultur. Landw. Vers. Sta. 30:18–41. 1884.
12. MARTIN, J. B. Microorganisms and soil aggregation. II. Influence of bacterial polysaccharides on soil structure. Soil Sci. 61:157–166. 1945.
13. NAGAOKA, M. On the behavior of the rice plant to nitrates and ammonium salts. Bull. Coll. Agr. Tokyo Univ. 6:285–334. 1904. (Exp. Sta. Rec. 16:560).
14. PARDO, J. H. Ammonium in the nutrition of higher green plants. Quart. Rev. Biol. 10:1–31. 1935.
15. RUSSEL, E. W. Soil structure. Imp. Bur. Soil Sci. Tech. Com. 37. 1938.
16. THELIN, G., and BEAUMONT, A. The effect of some forms of nitrogen on the growth and nitrogen content of wheat and rice plants. Jour. Amer. Soc. Agron. 26:1012–1017. 1934.
17. TRELEASE, S. F., and PAULINO, P. The effect on the growth of rice of the addition of ammonium and nitrate salts to soil cultures. Philippine Jour. Agr. 8:293–313. 1920.
18. VICKERY, W. B.; PUCHER, G. W.; WAKEMAN, A. S.; and LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. VIII. The effect on composition of the plant of the form in which nitrogen is supplied. Conn. Agr. Exp. Sta. Bull. 442. 1940.
19. WILLIAMS, W. R. Theses of tenacity and cohesion in soil structure. Pedology 5–6:755–762. 1935.

CHANGES WHICH OCCUR IN APPLE TISSUE UPON TREATMENT WITH VARIOUS AGENTS AND THEIR RELATION TO THE NATURAL MECHANISM OF SOFTENING DURING MATURATION[1]

JEAN H. GRIFFIN AND Z. I. KERTESZ

Introduction

Changes which occur in the texture and firmness of apples on the tree and in storage are of great economic importance. In order to make apples available to the consumer during extended periods, a great proportion of the crop is placed in storage in a "tree-ripe" or relatively firm condition. The rate of softening may then be controlled, or at least retarded, by refrigeration or by modifying the atmosphere within the storage (13) or both.

The firmness of an apple is one of the major factors which determines its commercial value. Changes in firmness during growth and maturation (5) may be followed by measuring the pressure necessary to force a plunger (10) into the apple (after careful removal of the skin), or by determining the tensile strength, i.e., the force required to pull to pieces a sample of tissue which has been cut according to standardized procedure (11). There are several reports in the literature of such measurements used as an index of maturation of apples; these methods are very useful where numerical recordings are desirable.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 679.

Over one hundred years ago it was observed that unripe apples contain pectic substances which are insoluble in water and that, as ripening progresses, an increasing proportion of water-soluble pectic substances (9) appears in the tissue (1, 2). The latter are apparently formed from originally insoluble pectic constituents which are usually designated as "protopectin" and "cell-wall pectic constituents." It has been the general experience among investigators that the softening of apple tissues, whenever it occurs or however it is induced, is accompanied by pectic transformations of such a nature.

It would be wrong to assume that changes in the pectic substances are entirely responsible for the changes in texture. The size and shape of cells and undoubtedly many other factors have an influence on the firmness of the tissue. In apples the formation of new cells stops comparatively early in development and at that time the individual cells are strongly cemented together by the middle lamella which is composed of insoluble pectic materials. During maturation the middle lamella is reduced in quantity; as a result, the adhesion of the cells to each other diminishes, and thus the tissue softens. It should be also borne in mind that chewing of a tissue consists mainly of pushing the cells (or clusters of cells) apart rather than of cutting the individual cells.

Ever since the chemical changes occurring during softening of apples were first observed, it has been assumed that transformations in the pectic substances were caused by the action of enzymes. In spite of the efforts of many workers, there is no conclusive evidence that protopectinase and pectin-polygalacturonase (pectinase) (6) actually occur in apples. During the last five years many tests have been made in this laboratory by various methods to show the presence of protopectinase and pectin-polygalacturonase (pectinase) in apples, but no results proving the presence of these enzymes could be obtained.

These negative results lead to the assumption that pectic transformations in apples may occur through the action of nonenzymatic agents or chains of reactions (8). It is known that polysaccharides, including pectinic acid (pectin), may be degraded by ascorbic acid (vitamin C) or peroxides, with an especially rapid reaction taking place when both agents are permitted to act simultaneously (4). Ascorbic acid is naturally present in apple tissue (though at times in small quantities), and peroxides have also been assumed to occur in plant tissues, although likely only in a transient state. Furthermore, peroxides were shown to form during the nonenzymatic oxidation of ascorbic acid (14); thus the presence of ascorbic acid may give rise to peroxides and produce the system for degrading pectinic acids.

Knowing that ascorbic acid and peroxides, alone or in combination, are capable of degrading pectinic acids (pectin) into products which no longer come within the definition of pectic substances, it appeared that possibly the pectic changes in apples during maturation might proceed, partly at least, through this mechanism. Of even more fundamental interest is the question whether or not these chemical agents are capable of transforming insoluble pectic constituents (protopectin) into soluble ones (pectinic acids and pectins). Since no method is available for the preparation of pure protopectin, experiments were performed with the apple tissue itself. Measurements of tensile strength, followed by analyses for soluble and in-

soluble pectic constituents, were made after treatments of the tissue with ascorbic acid, hydrogen peroxide, or both. In addition, some tests were made to determine the changes in firmness and chemical composition which were caused by treatment of apple tissue with dilute HCl solutions and with a commercial pectinase[2] known to contain high polygalacturonase (pectinase) activity.

Methods

Webster apples grown on one tree in Geneva, New York, during the 1944 season were used. Apples were picked fresh for each experiment until September 20, when all fruit was picked and put into storage at 1° C. After this date they were removed, as required, from cold storage and held at room temperature overnight before using. For measurement of tensile strength a cross-section, about 10 mm. thick, first was cut from the apple. From this disk dumbbell-shaped figures were cut out by a sharp form. These dumbbells were made to exact thickness (7.8 mm.) by placing them in a Petri dish and cutting with a sharp knife sliding along the edge of the dish. The dumbbells weighed 1.9 gm. and had a cross-section at the narrowest point, where breaking occurred, of 43.7 sq. mm. For the various treatments the dumbbells were submerged, usually in pairs, in 50 cc. of solution contained in a 100-cc. beaker. Every set of samples used for tensile-strength measurements or analyses consisted of twelve dumbbells, obtained from at least three apples. The tensile-strength measurements were performed with the aid of the apparatus described by PERSONIUS and SHARP (11). Water was siphoned into the bucket weighing 146 gm. at the rate of 3.2 gm. per second until the combined weight of the bucket and water tore the center of the dumbbell apart. At this time the water was shut off and the weight of the water plus bucket measured. The twenty-four halves of dumbbells were placed in ethanol, which was boiled up and then set aside for chemical analysis.

For analysis the sample (consisting of twelve dumbbells) was ground finely with approximately 12 gm. of sand and extracted with 200 cc. of water for 3 hours at about 20° C. The sample was then centrifuged at 2500 r.p.m. for 20 minutes in a no. 2 International Centrifuge, the extract poured off, and a second similar 30-minute extraction made. These two extracts were combined, made up to 500 cc. with water, and 200 cc. of this extract was then used for the precipitation of acid-ethanol-insoluble materials by a double volume of 95% ethanol acidified to give 0.05 *N* HCl in the final mixture. After standing overnight the precipitate was filtered off, washed with ethanol, dissolved in hot water, and the pectic substances determined as calcium pectate by the POORE modification (12) of the CARRÉ-HAYNES method (3).

The residue left from the water extraction was treated with 0.05 *N* HCl for 2 hours at 85° C. The liquid was filtered off and the process repeated four times. These five extracts were combined and made up to 1000 cc. with water. The pectic substances were then determined (as above) in 200 cc. of the extract after the removal, with ptyalin, of any starch which may have been present.

Results

The results of preliminary tests (table 1) indicate that the tensile strength of apple tissue decreases throughout the season. The proportion of pectic sub-

[2] Pectinol, manufactured by the Rohm and Haas Company of Philadelphia, Pa.

stances (on the fresh-weight basis) decreased continuously, although somewhat irregularly. The percentage of water-soluble pectic substances showed an irregular tendency to increase. When the apples were held at room temperature for a week, there was a considerable drop setting of the osmotic equilibrium in the tissue, resulting in some plasmolysis. As shown in tables 1 and 2, the effect on tensile strength of soaking in water alone was great enough to interfere with the clarity of observations on the effects of chemicals. The probable error of tensile-

TABLE 1

SEASONAL CHANGES AND EFFECT OF HOLDING AT ROOM TEMPERATURE OR SOAKING IN WATER ON TENSILE STRENGTH AND PECTIC CONSTITUENTS OF APPLES (WEBSTER VARIETY, 1944)

DATE	TREATMENT	TENSILE STRENGTH (GM./MM.²)	CHANGE IN TENSILE STRENGTH (%)	PROBABLE ERROR (GM./MM.²)	PECTIC SUBSTANCES†			
					Water soluble (%)	Water soluble (% of total)	Water insoluble (%)	Total (%)
8/15		21.0	(100)	±0.6				
8/28		20.0		±0.8				0.767
9/20*		17.5	(100)	±0.7	0.066	10.6	0.555	.621
9/27	In water for 60 min.	11.5	66					
	Held 1 week	13.3	76	±0.6				
9/21		19.5	(100)	±0.5				.756
	In water for 150 min.	16.8	86					.716
	In water for 300 min.	10.4	54					
9/27		15.6	(100)	±0.7	.056	9.1	.539	.614
10/5	In water for 165 min.	10.4	67	±1.0	.090	13.9	.557	.647
	Held 1 week	5.0	32	±0.3	.089	13.8	.559	.648
10/16		13.0	(100)	±0.5	.082	11.8	.612	.694
10/23	Held 1 week	3.3	25	±0.0	.107	20.0	.448	.535
10/17		5.8			.053	9.1	.529	.582
11/30		4.6			.100	17.6	.472	.572
12/15		3.7			0.087	15.6	0.469	0.556

* Apples picked and put into storage.

† As calcium pectate, fresh basis.

in tensile strength and an increase in the proportion of water-soluble pectic constituents.

Treatments by various agents were performed by submerging the apple dumbbells in the solutions. It was noted that soaking in water alone decreased the tensile strength. The reason for this change must be, partly at least, the up- strength measurements in samples soaked in water was higher than in any other lot of twelve dumbbells. When buffer solutions were used, tensile strengths of the dumbbells dropped much less than in water. The important point is, however, that the decrease of tensile strength in samples soaked in water was accompanied only by a small increase in the

proportion of soluble pectic substances, while the percentage of total pectic substances did not change materially.

Table 2 also shows some typical results obtained in tests made with ascorbic acid, hydrogen peroxide, pectinase, and HCl. In these experiments drop in tensile strength when both hydrogen peroxide and ascorbic acid were present. Although the proportion of total pectic constituents is irregular in these samples, those which were treated with both ascorbic acid and peroxide show a decrease.

TABLE 2

EFFECTS OF VARIOUS TREATMENTS ON TENSILE STRENGTH AND PECTIC SUBSTANCES OF APPLES (WEBSTER VARIETY, 1944)

Experiment no.	Date	Treatment*	Time (min.)	Tensile strength (gm./mm.²)	Probable error (gm./mm.²)	Pectic substances†			
						Water soluble (%)	Water soluble (% of total)	Water insoluble (%)	Total (%)
1.....	8/15	1. water	150	15.9	.....	.....	.....	.....	0.784
		2. H_2O_2 (1.5%)	"	15.1	.....	.....	.....	.....	.760
		3. AA (0.5%)	"	11.3	.....	.....	.....	.....	.855
		4. (2+3)	"	9.0	.....	.....	.....	.....	.524
		5. water	300	12.5	.....	.....	.....	.....	.751
		6. as 2	"	9.3	.....	.....	.....	.....	.718
		7. as 3	"	10.2	.....	.....	.....	.....	.807
		8 as 4	"	4.6	.....	.....	.....	.....	.467
2.....	9/1	Water	150	16.8	.....	0.124	17.8	0.592	.716
		0.001 *N* HCl	"	12.6	.....	.117	16.7	.583	.700
		0.01 *N* HCl	"	8.9	.....	.....	.....	.....	.....
3.....	9/27	(Control)	.....	15.6	±0.7	.056	9.1	.539	.614
		1. water	165	10.4	±1.0	.090	13.9	.557	.647
		2. Buffer pH 3.3	"	12.9	±0.8	.102	13.7	.645	.747
		3. E (0.25%)	"	6.4	±0.6	.097	16.7	.483	.580
		5. AA (0.1%)	"	8.7	±0.6	.167	29.9	.492	.559
		6. AA (0.05%)	"	9.7	±0.7	.154	24.9	.465	.619
		7. H_2O_2 (3%)	"	9.4	±0.6	.259	44.0	.330	.589
		8. H_2O_2 (1.5%)	"	10.6	±0.7	.229	37.7	.379	.608
		9. (5+8)	"	7.2	±0.4	.228	50.6	.223	.451
		10 (6+7)	"	7.7	±0.4	.209	38.8	.330	.539
		11. (5+7)	"	6.8	±0.4	.170	32.6	.351	.521
		12. (6+8)	"	7.5	±0.4	0.160	31.1	0.355	0.515

* AA=ascorbic acid; E=commercial pectic enzyme (pectinase).

† As calcium pectate, fresh basis.

the treated dumbbells were compared with those soaked in water or, in the last experiment (no. 3), in water and buffer solution (pH 3.3) for corresponding periods.

The first experiment showed a definite

In order to ascertain just how much the acidity of the ascorbic acid may have affected the tensile strength and the total proportion of pectic constituents, some dumbbells were soaked in solutions of hydrochloric acid. As shown in experi-

ment 2 (table 2), concentrations corresponding in pH (even if not in ionic strength) to the ascorbic acid solutions used caused some change in tensile strength but no change in distribution and total quantity of pectic constituents.

The most important set of data is that obtained in experiment 3. A 0.1 *M* phosphate buffer solution was used in all these mixtures to maintain the pH at 3.3. The use of this buffer resulted in 17% less drop in tensile strength than when water was used for the soaking (33% drop). All other solutions were made in such buffer, and all mixtures were adjusted to pH 3.3.

The enzyme treatment lowered the total pectic content, with an accompanying increase in soluble pectic constituents. Every treatment with ascorbic acid, hydrogen peroxide, or both, increased the proportion of soluble pectic constituents and decreased the percentage of total pectic constituents. These changes were in every instance accompanied by a decrease in tensile strength of the apple tissue. The drop in tensile strength and the drop in total pectic constituents are greatest where both ascorbic acid and hydrogen peroxide were used simultaneously and approximate the results obtained when the commercial pectic enzyme was used.

Discussion

The results of several investigators (4, 8) proved that ascorbic acid or peroxides, and especially ascorbic acid and peroxides together, can rapidly degrade soluble pectic compounds like pectinic acids (pectins). The relationship between protopectin, the major insoluble pectic constituent of plants, and soluble pectic substances is still not clear, but there is a distinct possibility that protopectin is rendered soluble by the action of heat, acids, or enzymes through a partial hydrolysis which would diminish its originally large molecular weight (size) (7). Treatment of insoluble pectic constituents of apples with hydrogen peroxide and ascorbic acid results in an increase in soluble pectic substances in the apple tissue. The soluble pectic constituents are then further degraded into compounds which will no longer come under the definition of pectic substances. It seems that in our experiments the latter reaction was slower than the rate of solubilization of the insoluble protopectin, since both the absolute amount and the proportion of soluble pectic constituents increased. Unfortunately, the mechanism of oxidative degradation by ascorbic acid and peroxides is yet so unclear that further speculation on these reactions is hardly warranted.

Obviously, the treatments used in these experiments resemble only to a limited degree those processes which may occur naturally in the plant. Concentrations of ascorbic acid and peroxide used in the experiments could not be assumed to be present in the fruit tissue. However, the purpose of the experiments was not an imitation of the changes *as* they occur in nature but rather to determine whether the changes which take place in the pectic constituents of apples during growth and maturation *could be* artificially brought about by the nonenzymatic agents used. According to the results obtained, this has been accomplished. As apples soften, the total pectic constituents decrease and the proportion of soluble pectic materials increases. Similar changes have been brought about in the tissue by the use of ascorbic acid, peroxide, or both. In nature these transformations occur during periods of weeks or months rather than

hours and thus might require much lower concentrations of the chemical agents than those used in the tests.

Summary

1. During the maturation and softening of apples the tensile strength of the tissue and the proportion of the total pectic constituents decrease and the proportion of water-soluble pectic materials increases. The same changes may be brought about by treatment of apple tissue with ascorbic acid or hydrogen peroxide and at a much faster rate by a combination of both agents.

2. The results indicate that, in addition to being able to decompose water-soluble pectinic acids (pectins), these agents are also able to solubilize the insoluble pectic constituents (protopectin) of apples.

3. While it appears that both protopectinase and polygalacturonase (pectinase) are absent from apples, the functions attributed to both enzymes may be performed by the nonenzymatic agents used in these experiments.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION
CORNELL UNIVERSITY
GENEVA, NEW YORK

LITERATURE CITED

1. BRANFOOT, M.A. A critical and historical study of the pectic substances of plants. British Food Investigation Board Special Report No. 33. 1929.
2. CARRÉ, M. H. The relation of pectase and pectin in apple tissue. Biochem. Jour. 19:257–265. 1925.
3. CARRÉ, M. H., and HAYNES, D. The estimation of pectin as calcium pectate and the application of this method to the determination of soluble pectin in apples. Biochem. Jour. 16:60–69. 1922.
4. DEUEL, HANS. Oxydativer Abbau von Pektin in wässeriger Lösung. Viscosimetrische Bestimmungen. Helvetia Chim. Acta 26:2002–2025. 1944.
5. HALLER, M. H. Changes in pectin constituents of apples in relation to softening. Jour. Agr. Res. 39:739–746. 1929.
6. KERTESZ, Z. I. Pectic enzymes. Ergebnisse der Enzymforsch. 5:233–258. 1936.
7. ———. Note on the nature of protopectin and interrelation of pectic substances. Paper given at Memphis meeting, American Chemical Society, April, 1942. (Unpublished.)
8. ———. Possible non-enzymatic mechanism of changes in the pectic substances and other polysaccharides in living plants. Plant Physiol. 18: 308–309. 1943.
9. KERTESZ, Z. I.; BAKER, G. L.; JOSEPH, G. H.; MOTTERN, H. H., and OLSEN, A. G. Report of the Committee for Revision of Nomenclature of Pectic Substances. Chem. and Eng. News 22: 105–106. 1944.
10. MAGNESS, J. R., and TAYLOR, G. F. An improved type of pressure tester for the determination of fruit maturity. U.S.D.A. Circ. 350. 1925.
11. PERSONIUS, C. J., and SHARP, P. F. Permeability of potato-tuber tissue as influenced by heat. Food Research 3:513–524. 1938.
12. POORE, H. D. Recovery of naringin and pectin from grapefruit residue. Ind. and Eng. Chem. 26:637–639. 1934.
13. SMOCK, R. M. Physiology of deciduous fruits in storage. Bot. Rev. 10:525–598. 1944.
14. STEINMANN, H. G., and DAWSON, C. R. On the mechanism of the ascorbic acid–ascorbic acid oxidase reaction. Jour. Amer. Chem. Soc. 64: 1212. 1942.

PRELIMINARY RESULTS WITH THE USE OF 2,4-DICHLOROPHENOXYACETIC ACID AS A SPRAY-OIL AMENDMENT[1]

WILLIAM S. STEWART[2] AND WALTER EBELING[3]

Introduction

Spraying citrus trees with emulsions of oil in water is a standard practice in citrus-pest control. Such sprays are very effective and offer cost advantages over other methods of insect control. Oil sprays, however, may induce leaf and fruit drop (4). Die-back of small twigs, granulation, and water spot (1, 2, 5) are a few of the other indictments against the use of oil sprays.

In general, there is an inverse correlation between the unsulfonated residue (U.R.) of the spray oil (4) and the amount of leaf drop, fruit drop, and die-back of small twigs. The higher the unsulfonated residue, other factors being equal, the fewer these undesirable effects. For this reason the percentage of unsulfonated residue in the oil has become an important specification of spray oils. Oils now used in California are 90–94 U.R., but even at these values leaf and fruit drop often occur to an undesirable extent after a single annual spraying.

Plant growth-regulators are known to be very effective in preventing preharvest fruit drop of certain varieties of apples (8). These substances have also been shown to prevent leaf drop from cut branches of several plant species (9, 11), and their use to decrease fruit drop in oranges has been indicated (6). These facts led to consideration of the use of plant growth-regulators as spray-oil amendments to limit the undesirable effects of oil sprays. The present paper reports preliminary observations on reduction in leaf and fruit drop as the result of adding plant growth-regulators to spray oil used on Washington Navel and Valencia orange trees.

[1] Paper no. 547, University of California Citrus Experiment Station, Riverside, Calif.

[2] Assistant Plant Physiologist and [3] Associate Entomologist, California Agricultural Experiment Station.

Experimentation

Exploratory tests

Mature leaves on a Washington Navel orange tree were painted with undiluted spray oil with or without 0.1% 2,4-dichlorophenoxyacetic acid (2,4-D). In this as in all subsequent work, the oils used corresponded to the California grade "light-medium" (52–61% distilled at 636° F.). Table 1 lists other properties of these oils. To accentuate leaf drop, 60 (instead of 92) U.R. oil was used. The leaves painted with oil containing 0.1% 2,4-D were more securely attached to the stem after 10 days than were those painted with oil alone. Repeated tests gave similar results.

Next, two branches on the same tree were given a drenching spray with the undiluted oil, with and without 0.1% 2,4-D. Application was made with a 1-quart continuous-pressure garden sprayer. Two weeks after spraying there was a striking difference between the two branches, the one treated with 2,4-D retaining most of its leaves and fruit, in contrast to the other, which was completely defoliated and had lost most of its fruit (fig. 1). This test was repeated twice with the same result.

Additional, comparable tests were made, using 2,4-D, as well as phenoxyacetic acid, naphthoxyacetic acid, naphthaleneacetic acid, and naphthalene acetamide, singly and in combination with 2,4-D. In these tests, as in others in which various growth-regulating substances were used to limit preharvest drop in certain varieties of apples (3), 2,4-D alone was found to be the most effective.

The minimum effective concentration of 2,4-D, in limiting leaf drop, in oils of 60, 71, 80, and 92 U.R. was also determined. Concentrations of 0.005, 0.025, 0.05, and 0.1% 2,4-D were prepared in each oil by dissolving 2,4-D in ethyl alcohol and evaporating off the alcohol after addition to the oil. The undiluted oils were sprayed, as described, on single branches. A separate tree was used for each concentration of 2,4-D in each oil—a total of sixteen trees. For comparison, a second, similar branch on each tree

TABLE 1

PHYSICAL PROPERTIES OF THE LIGHT-MEDIUM NAPHTHENIC OILS USED IN PREPARING OIL-WATER SPRAY EMULSIONS

Oil no.	Unsulfonated residue (%)	Temperature (° F.) for distillation of		Saybolt viscosity at 100° F. (secs.)	Gravity (60°/60° F.)	Refractive index (n_D^{20})
		5%	90%			
1	60	570	698	75	0.925	1.516
2	71	443	705	48	.887	1.490
3	80	501	703	55	.883	1.485
4	92	571	695	71	0.886	1.482

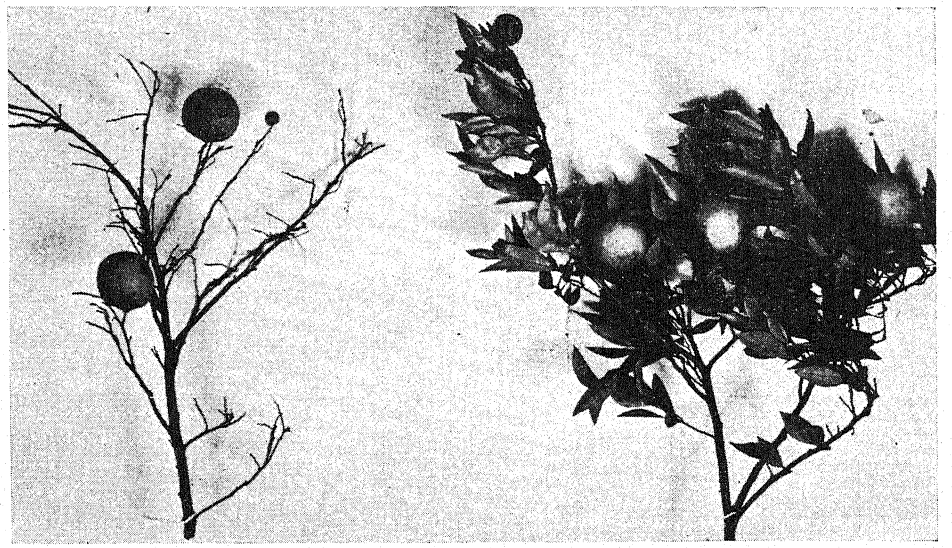


FIG. 1.—Branches of Washington Navel orange tree, showing effect on leaf and fruit drop of applying 60 U.R. oil spray (*left*) undiluted and (*right*) with 0.1% 2,4-dichlorophenoxyacetic acid added.

was sprayed with the same oil lacking 2,4-D.

Concentrations of 0.025% 2,4-D, or higher, in all the oils, were very effective in limiting leaf drop. The effects were similar to those shown in figure 1. No differences in leaf drop were noted between control branches (sprayed with oil only) and those treated with 0.005% 2,4-D in oil. The 0.05% treatment was more effective in limiting leaf drop than the 0.025%; there were no apparent differences between the 0.05% and 0.1% 2,4-D treatments. In these trials all concentrations of 2,4-D in oil were equally effective in decreasing fruit drop by 50%, compared with that on branches sprayed with oil alone. It is of interest that the most effective concentration for delaying petiole abscission in *Coleus*, in tests with a large number of growth-regulators, was found to be 0.05% (7).

On the basis of these data, three field trials were made.

FIELD TEST NO. 1

PROCEDURE.—This test was with Washington Navel orange trees in an orchard at Riverside, California, and consisted of a Latin-square arrangement of five treatments. Because of the size and shape of the orchard, a sixth treatment could not be included in the square but was made on adjacent trees. All trees had previously been sprayed with oil, in April, 1945, and the fruit had been picked in December of that year.

Treatments in the square were: (1) no oil spray (control); (2) 92 U.R. oil spray, at 2% concentration in water; (3) same as 2, but with 0.005% 2,4-D in the oil; (4) same as 2, but with 0.025% 2,4-D added; (5) 80 U.R. oil spray, at 2%, containing 0.05% 2,4-D. Treatment 6, outside the square, was the same as 5, except that 2,4-D was omitted. Since the relative solubility of 2,4-D in oil and water is not known, the concentration of 2,4-D is expressed on the basis of its concentration in the oil rather than in the final oil-water emulsion. If 2,4-D were as soluble in water as in oil, its concentration on the emulsion basis would be 0.001% (10 p.p.m.) for the 0.05% 2,4-D in oil used in the 2% oil emulsion.

All spray emulsions were tank-mixed, using 4 ounces of blood albumin per 100 gallons of spray as spreader. The 2,4-D was first dissolved in ethyl alcohol and was then added to the oil as described. The spray was applied in the usual manner on February 18 and 19, 1946, with a power sprayer, using 400 pounds pressure and no. 8 disks. Contamination of control trees by drifting 2,4-D spray was prevented by completely covering them with canvas tents when spraying near by. All leaves were raked from under the trees at the beginning of the experiment.

In this trial, as in all field trials, leaf-drop counts were made by determining the number of leaves within a rectangular frame approximately 1 × 10 feet. This frame was always placed in a southeasterly direction against the tree trunk and extended several feet out beyond the drip of the tree.

RESULTS.—One week after application, trees sprayed with 80 U.R. oil alone (treatment 6) had dropped many more leaves than those sprayed with the same oil containing 0.05% 2,4-D (treatment 5). There was a similar difference between the unsprayed (control) trees and the oil-sprayed ones. At this time leaf-drop differences were not obvious between the trees sprayed with 92 U.R. oil with and without 2,4-D.

To determine whether 2,4-D had induced any differences in leaf drop, five leafy twigs per treatment were cut from

the trees 1 week after spraying and were placed in an atmosphere of ethylene and high humidity in the laboratory. Forty-eight hours later, under these conditions, the total numbers of leaves remaining on each set of five twigs were 0, 1, 5, 10, 18, and 5, respectively, for treatments 1 through 6. These results indicate the effectiveness of treatments 3 and 4 (0.005 and 0.025% 2,4-D in oil) in limiting leaf drop on trees sprayed with 92 U.R. oil, although differences were not yet apparent in the field.

The obvious difference observed in the field between treatments 5 and 6 (80 U.R. oil with and without 0.05% 2,4-D) was also shown in a laboratory ethylene treatment one week after spraying. However, a similar laboratory test made approximately 18 hours after spraying showed no differences between the oil-sprayed leaves with or without 2,4-D. It appears that 2,4-D in oil became effective in limiting leaf drop between 18 and 168 hours after application.

Leaf-drop counts in the field on March 5, over 2 weeks after spraying, showed that addition of 2,4-D to the spray oil resulted in a significant decrease in leaf drop, in comparison with that of trees sprayed with the same oil without 2,4-D (table 2). With 92 U.R. oil containing 0.025% 2,4-D, the decrease was 26.5%; with 80 U.R. oil with 0.05% 2,4-D, the decrease was 57.4%. Even with the addition of 2,4-D, however, the 92 U.R. oil sprays induced about three times as much leaf drop as in unsprayed trees. Leaf-drop counts on March 25 showed comparable differences. Fruit-drop observations on these trees were not possible, as the fruit had been harvested.

Field test no. 2

Procedure.—This test, with Washington Navel orange trees in an orchard at La Verne, California, consisted of a Latin-square arrangement of four treatments: (1) no oil spray (control); (2) 92 U.R. oil spray, at 1.66% concentration; (3) same as 2, but with 0.0037% 2,4-D added to the oil; and (4) same as 2, but with 0.0185% 2,4-D added to the oil.

The required amounts of 2,4-D were added as a commercial liquid concentrate of 40% 2,4-D as the diethanol ammonium salt, instead of the 2,4-D free acid being dissolved in alcohol as in the previous trial.

To avoid effects of spray drift, the treated trees were separated from one another by two trees in each direction. The trees were about 35 years old and had been fumigated but had never before been sprayed with oil. The sprays were applied February 20, 1946, when the fruit on the trees was mature. All leaves and fruit on the ground under the trees were removed.

Although the trees were as uniform as could be expected, there was still variation in size. It was suggested that leaf drop might be correlated with tree size and that a correction for tree volume would be desirable. An analysis of covariance (10) gave a nonsignificant F value, indicating that it made no difference whether leaf drop was corrected for tree volume.

Results.—The numbers of leaves dropped per tree by March 5, 1946, for treatments 1 through 4, were 40, 211, 296, and 162, respectively (table 2). The addition of 0.0185% 2,4-D to oil applied at 1.66% concentration in the emulsion did not significantly limit leaf drop. Counts on March 26 likewise failed to show a statistically significant difference in leaf drop of trees sprayed with oil plus 2,4-D, in comparison with those sprayed with oil alone.

Fruit drop at La Verne for the period

March 21–April 25, from the trees sprayed with 0.0037% 2,4-D oil amendment, was 32.4% less than that from trees sprayed with oil only (table 2). This difference was significant at the 5% level. The decrease in fruit drop for the spray containing 0.0185% 2,4-D was 30.9%. The unsprayed trees dropped 32.8% less fruit than those sprayed with oil alone. Fruit loss induced by the oil spray was thus counteracted by the addition of 2,4-D.

Fruit-drop counts prior to March 21 showed a trend similar to that reported above, but the differences lacked significance at the 5% level. This may indicate that 2,4-D applied in oil required approximately a month to become effective in limiting fruit drop. GARDNER (6), for example, found that naphthaleneacetic acid and naphthalene acetamide were most effective in decreasing drop of Pineapple oranges in Florida when applied several months before harvest.

TABLE 2

EFFECT OF OIL SPRAY, WITH AND WITHOUT ADDED 2,4-DICHLOROPHENOXYACETIC ACID, ON LEAF AND FRUIT DROP OF WASHINGTON NAVEL AND VALENCIA ORANGE TREES

Treatment no.	Spray oil		% 2,4-D in oil	No. trees	No. leaves dropped	No. fruits dropped
	% U.R.	% in emulsion				
	Field test no. 1 (Washington Navels, Riverside)*					
1 (control)	.....	.....	.....	5	94	.....
2	92	2.00	0.0000	5	411	.....
3	92	2.00	.0050	5	397	.....
4	92	2.00	.0250	5	302	.....
5	80	2.00	.0500	5	571†	.....
6	80	2.00	0.0000	5	1341	.....
	Field test no. 2 (Washington Navels, La Verne)‡					
1 (control)	.....	.....	.....	4	40	120.2
2	92	1.66	0.0000	4	211	179.0
3	92	1.66	.0037	4	296	120.7
4	92	1.66	0.0185	4	162	123.6
	Field test no. 3 (Valencias, Riverside)					
1 (control)	.....	.....	.....	8	74	.....
2	92	2.00	0.0000	8	402	.....
3	92	2.00	.0050	4	369	.....
4	92	2.00	.0250	4	283§	.....
5	80	1.50	.0000	2	521	.....
6	80	1.50	0.0500	2	400	.....

* Least significant difference at 5% level, 102 leaves.
† Compared with same spray without 2,4-D, difference significant at 1% level. (Treatment not in Latin square.)
‡ Least significant difference at 5% level: leaves, 132; fruits, 58.
§ Compared with same spray without 2,4-D, difference significant at 5% level. (Treatment not in Latin square.)

A concentration of 0.0037% 2,4-D in oil was effective in limiting fruit drop, whereas the lowest effective concentration for a significant decrease in leaf drop seems to be between 0.0185% and 0.025%. This is a concentration at least five times greater than that required for a significant decrease in fruit drop. This result is in agreement with the observation by GARDNER and COOPER (7) that "the prevention of leaf abscission appears to require a much higher concentration of applied growth substance than the prevention of mature fruit drop."

FIELD TEST NO. 3

PROCEDURE.—This test was with Valencia orange trees in an orchard at Riverside, California. The trees were sprayed on February 22, 1946. The six treatments were: (1) no oil spray (control); (2) 92 U.R. oil spray, at 2% concentration in water; (3) same as 2, but with 0.005% 2,4-D added to the oil; (4) same as 2, but with 0.025% 2,4-D added; (5) 80 U.R. oil spray, at 1.5% concentration in water; (6) same as 5, but with 0.05% 2,4-D added to the oil.

There was at least one untreated tree (usually there were two) between the trees in treatments 1, 2, and 5 (no 2,4-D used) and the trees in treatments 3, 4, and 6 (sprayed with 2,4-D in oil). Except for this restriction, the treatments were randomized among trees in two rows. The numbers of trees used for treatments 1 through 6 were: 8, 8, 4, 4, 2, and 2, respectively. Maturing fruit was on the trees. Oil emulsions and 2,4-D additions were prepared and applied as in the La Verne trials.

RESULTS.—Counts on March 9 indicated a 29.6% decrease in leaf drop (significant at the 1% level) owing to the addition of 0.025% 2,4-D to the 92 U.R. oil (table 2). With the 80 U.R. oil, the decrease in leaf drop resulting from the addition of 0.05% 2,4-D was 23.2%. Since only two trees were available for each treatment with the 80 U.R. oil, the significance of this decrease could not be statistically tested.

Leaf-drop determinations on March 25 showed differences similar to those above. There was no obvious drop of the immature fruit as a result of the oil spray. It will be of interest to note whether 2,4-D oil spray applied to trees with immature fruits will decrease the drop of the mature fruits.

The above data indicate that the positive results in the second and third field tests are due to 2,4-D and not to the diethanolamine in which it was added, since they are the same as in the previous trials in which no diethanolamine was used.

TREE INJURY

The possibility of more tree damage from 2,4-D than from the oil spray is a consideration of prime importance. It has been generally observed that 2,4-D in water solutions of 0.1% has a deforming effect on expanding leaves and stems of the orange tree. In July, 1945, two 10-year-old Valencia orange trees with no expanding leaves or buds were sprayed with 2,4-D (approximately 0.14%) in water. Until November of that year, no effects of the spray were apparent, except for a lack of new growth; owing to the location of the trees, it was difficult to determine whether this was the result of 2,4-D injury. Several trees sprayed at the same time with 0.07% 2,4-D in water also failed to show new growth. Valencia trees sprayed September 15, 1945, with 0.14% 2,4-D in water showed epinastic curvatures of the actively growing stems and leaves and a yellowing of the expanding leaves. The plant parts that were not

expanding made no externally visible response. In all cases observation of the roots disclosed no indications of injury. Since all these trees were destroyed when the tract was cleared, in November, 1945, additional observations were not possible. On other trees left standing in the same grove, on which only isolated actively growing branches had been sprayed in September, 1945, the effects described above were still persistent in April, 1946. No lateral buds were then expanding on the sprayed branches.

A 15-year-old grapefruit tree sprayed September 15, 1945, with 0.14% 2,4-D in water, responded in the same manner as the oranges. The shoots that were actively growing at the time of spraying were still distorted in April, 1946, and showed no tendency to become normal. This response and the apparently delayed opening of the flower buds were the only obvious effects of 2,4-D. The roots showed no changes because of the treatments and appeared to be healthy.

A 5-year-old lemon tree with many actively growing shoots was sprayed at the same time (September 15) and in the same manner as the grapefruit. Severe epinastic curvatures of the growing stems and leaves occurred within 10 days. After one month, the pedicels on sprayed trees were thickened in comparison with those on nonsprayed trees, and the fruit appeared to be very securely attached. By December many apparently mature leaves had become a dull-yellow color.

As expected from the above observations, the stage of bud expansion appears to be an important factor in the use of 2,4-D as a spray-oil amendment. At the time of spraying the Riverside Navel plot (field test no. 1) many stem and flower buds had expanded to a length of 2 mm., and young leaves were 3–7 mm. long. Three weeks after spraying with 0.025% 2,4-D, the lateral halves of the basal portions of the leaf blades were expanding normally but the apical halves remained closed. By April 15, however, most blades had opened normally their entire length, and none remained tightly closed. The expanding leaves on the trees sprayed with 0.05% 2,4-D curled tightly inward and had failed to open in the 2 months after spraying. By July, when the next growth flush occurred, these leaves were expanded fully, except for a slight pinching-together of the blade near the tip. No effect on leaf growth was observed from the 0.005% 2,4-D spray. Opening of flower buds was apparently normal in all treatments.

In contrast to the Riverside Navels, the Riverside Valencias and the La Verne Navels were in a tighter bud stage at the time of spraying and, in general, the expanding vegetative and flower buds showed no undesirable responses, even to the 0.05% 2,4-D spray. Only rarely were the above-described effects observed on these trees. Possibly the few scattered buds showing a response were further expanded than the others at the time of spraying.

To avoid the deforming effects resulting from sprays containing 2,4-D, application should be made only when the tree has a minimum of actively growing branches and not during a growth flush. In general, this corresponds with the times when sprays are usually applied. These observations all forcibly emphasize the fact that the use of 2,4-D as a spray-oil amendment must await further study of undesirable effects on the trees. Such studies are now in progress.

Discussion

The addition of 0.025% 2,4-D to 92 U.R. tank-mixed oil used at 2% concentration in water on Washington Navel and Valencia orange trees at Riverside (table 2) significantly limited leaf drop,

in comparison with results from the same oil spray used without 2,4-D. The use of a similar spray oil at 1.66% concentration, with 0.0185% 2,4-D, on Washington Navels at La Verne, also decreased leaf drop, but the difference between these results and those obtained with the same oil spray without 2,4-D was not statistically significant. The difference in the results at Riverside and La Verne was apparently not caused solely by the lower concentration of 2,4-D used at La Verne but was partly the result of the smaller oil deposit on the leaves in the La Verne test, owing to the lower percentage of oil in the emulsion; this was indicated by direct determinations of the amount of spray oil deposited on the leaves.

Duplicate analyses for deposited oil were made on 100 leaves per treatment, collected equally from all trees in the treatment on the day following spraying. For treatments 2 (92 U.R. oil only, at 2%) and 4 (the oil plus 0.0185% 2,4-D), the amounts of deposited oil on the Navel orange leaves from La Verne were, respectively, 65.8 and 53.3 ml. $\times 10^{-6}$/sq. cm. of leaf area. For the corresponding treatments on the Valencia leaves from Riverside, the amounts of deposited oil were, respectively, 69.1 and 83.6 ml. $\times 10^{-6}$/sq. cm. The Valencia trees showed a significant decrease in leaf drop, even though there was actually more oil deposited on the leaves of the trees sprayed with oil plus 2,4-D than on those sprayed with oil alone. In the La Verne trials the comparison failed to show a significant decrease in leaf drop, even though in this case less oil was deposited on the leaves treated with 2,4-D than on those with oil alone.

Assuming that all the 2,4-D added was carried in the oil in the emulsion, the maximum amounts of 2,4-D per square centimeter of leaf surface of the Riverside Valencias and La Verne Navels may be calculated to have been 0.0209 μ gm. and 0.0099 μ gm., respectively. The amount of 2,4-D actually effective in the La Verne trial was 53% less than in the Riverside trial, instead of 26% less, as it would have been on the basis of 2,4-D concentration alone. If the upper and lower leaf surfaces are assumed to total 50 sq. cm., the amount of 2,4-D deposited per leaf on the Valencia trees was 1.04 μ gm. Since this amount is probably very small compared with that of the exploratory trials, where branches were sprayed with undiluted oil with 2,4-D added, tests are being made with oil-emulsion sprays containing higher concentrations of 2,4-D than those reported here.

Although the foregoing comparison is between two varieties of oranges (Washington Navel and Valencia), the observed differences in leaf drop because of varietal differences are not considered significant here, since at Riverside both varieties gave the same response to the same treatment.

Summary

1. Exploratory tests indicated that the addition of 0.025% 2,4-dichlorophenoxyacetic acid to undiluted spray oil applied to branches of both Washington Navel and Valencia orange trees strikingly limited leaf and fruit drop, in comparison with that of trees similarly sprayed, but without 2,4-D.

2. In preliminary field trials the addition of 0.025–0.05% 2,4-D to the oil in oil-water emulsion sprays for use on orange trees significantly decreased leaf drop 26.5% to 57.4%, the percentage reduction depending on the properties of the oil and on the concentration of 2,4-D.

3. In the one trial where fruit counts were possible, the addition of 0.0037% 2,4-D to the spray oil significantly de-

creased fruit drop 30.9%, compared with drop from trees similarly sprayed, but without 2,4-D.

4. Experiments have shown that the stage of bud expansion appears to be an important factor in the use of 2,4-D as a spray-oil amendment. At the present time, 2,4-D as a spray-oil amendment cannot be recommended for commercial orchard practice, since under certain conditions not yet fully established injury to trees may result from its use.

The authors thank the California Spray Chemical Company of Pittsburg, California, and the Thompson Horticultural Chemicals Corporation of Los Angeles, respectively, for supplying spray oils and plant growth-regulators. They appreciate, also, the generous co-operation of Mr. M. A. McDERMONT of Riverside and Mr. E. GRAHAM of the Peyton Corporation, La Verne, in making trees available for these studies.

The able assistance of Mr. J. WOOD and Mr. H. G. JOHNSON in the field studies, and of Mr. J. P. LADUE in making the oil-deposit determinations, greatly facilitated this work.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BARTHOLOMEW, E. T. The effect of oil sprays on plant tissues. West. Shade Tree Conf. Proc. 3:53–61. 1936.
2. BARTHOLOMEW, E. T., and SINCLAIR, W. B. Granulation and juice quality in Valencias as affected by insecticides. California Citrograph 30:4–5. 1944.
3. BATJER, L. P., and MARTH, P. C. New materials for delaying fruit abscission of apples. Science 101:363–364. 1945.
4. DE ONG, E. R.; KNIGHT, H.; and CHAMBERLIN, J. C. A preliminary study of petroleum oil as an insectide for citrus trees. Hilgardia 2:351–384. 1927.
5. EBELING, WALTER, and KLOTZ, L. J. The relation of pest-control treatment to water rot of Navel oranges. California Dept. Agr. Monthly Bull. 25:360–368. 1936.
6. GARDNER, F. E. Practical applications of plant growth-substances in horticulture. Florida State Hort. Soc. Proc. 54:20–26. 1941.
7. GARDNER, F. E., and COOPER, W. C. Effectiveness of growth substances in delaying abscission of *Coleus* petioles. BOT. GAZ. 105:80–89. 1943.
8. GARDNER, F. E.; MARTH, P. C.; and BATJER, L. P. Spraying with plant growth-substances to prevent apple fruit dropping. Science 90:208–209. 1939.
9. MILBRATH, J. A., and HARTMAN, HENRY. Holly defoliation prevented by α-naphthaleneacetic acid. Science 92:401. 1940.
10. SNEDECOR, G. W. Statistical methods. Iowa State College Press, Ames, Iowa. 1940.
11. WORLEY, C. L., and GROGAN, R. G. Defoliation of certain species as affected by alpha-naphthaleneacetic acid treatment. Jour. Tenn. Acad. Sci. 16:326–328. 1941.

ANALYSIS OF LEVULINS, INULIN, AND MONOSACCHARIDES IN GUAYULE[1]

HAMILTON P. TRAUB[2] AND M. C. SLATTERY[3]

Introduction

In previous papers the nature of the water-soluble carbohydrates in guayule (*Parthenium argentatum* A. Gray) has been established (2, 5, 11, 12), and the importance of relating the relative proportions of the 89%-ethanol-soluble and the 89%-ethanol-insoluble levulins to plant functioning has also been indicated (11). The reader is referred to TRAUB and SLATTERY (11) for criticism of the earlier untenable conception of the nature of the chief carbohydrate reserve in guayule (4). In the present paper methods of extracting and analyzing for water-soluble carbohydrates and for distinguishing between 89%-ethanol-soluble and 89%-ethanol-insoluble levulins are briefly summarized. Typical results obtained by the application of these methods, showing seasonal changes in guayule tissues, are presented.

Methods

EXTRACTION OF TISSUES

The tissue samples are dried, finely ground, and then again dried at 65° C., according to the procedure described by TRAUB (9). The method for the extraction of water-soluble carbohydrates is adapted from the work of TRAUB *et al.* (13) and consists of (*a*) cold-water extractions for the determination of total free sugars, total free fructose, and total cold-water-soluble carbohydrates (free sugars plus levulins) and of (*b*) hot-water extractions for the determination of total water-soluble carbohydrates (free sugars, levulins, and inulin).

COLD-WATER EXTRACTION.—Duplicate 2-gm. samples of the finely ground tissue, prepared as described by TRAUB (11), are transferred to 100-ml. volumetric flasks, provided with glass stoppers, by means of a stemless glass funnel. Approximately 1 gm. of powdered $CaCO_3$ and 50 ml. of water are then added, and the tissue is wetted by rotating the flasks, which are then placed in a water bath at 20° C. for 1 hour. After removal, 1 ml. of a saturated solution of neutral lead acetate is added, and the contents of the flasks are made up to volume and thoroughly mixed. After standing for a few minutes to allow for complete precipitation of proteins, tannins, etc., the contents are filtered into flasks with 0.2–0.3 gm. of dry powdered sodium oxalate on the bottom. After shaking, the contents of the flasks are allowed to stand for a few minutes to insure complete deleading and are then filtered into 125-ml. dry Erlenmeyer flasks. Aliquots of the filtrate are used for the determination of total free sugars, total free fructose, and total cold-water-soluble carbohydrates (free sugars plus levulins).

HOT-WATER EXTRACTION.—The pro-

[1] Contribution from the Emergency Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture. The co-operation of the U.S. Forest Service is gratefully acknowledged.

[2] Principal Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Plant Industry Station, Beltsville, Md.

[3] Formerly Assistant Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering.

cedure is the same as that outlined in the preceding paragraph except that the flasks containing the wetted tissue are placed in a boiling-water bath for 1 hour, and the neutral lead acetate is added after cooling the contents of the flasks to 20° C. in a water bath. Aliquots of the final filtrate from these extractions are used for the determination of total water-soluble carbohydrates (free sugars, levulins, and inulin).

At times considerable difficulty may be encountered in filtering hot-water extracts of guayule leaves and new growth, apparently because of the colloidal state of some of the constituents extracted with hot water. In such cases, one or two spoonfuls of diatomaceous earth are placed on the filter paper before the plant extracts are poured on.

Carbohydrate analysis

Aliquots from the cold-water extractions, without hydrolysis, are used for the determination of total free sugars by Somogyi's semimicro copper method (8) as modified by Browne and Zerban (1) and of total free fructose by Nyn's cupro-carbonate method (6) as modified by Jackson and Mathews (3).

Levulins, or levulins plus inulin, in 10-ml. aliquots from cold- or hot-water extractions, are hydrolyzed with 1 ml. of 10% HCl (1.0741 sp. gr.) in a hot-water bath at 70° C. for 10 minutes. The solutions are neutralized with dilute NaOH, with phenolphthalein as indicator. Aliquots are then taken for the determination of total cold-water-soluble carbohydrates (free sugars and inulin) and total water-soluble carbohydrates (free sugars, levulins, and inulin), respectively, again by Somogyi's semimicro copper method (8) as modified by Browne and Zerban (1).

For the rapid determination of total fructose (free and polymerized) in the cold- or hot-water extracts, Roe's alcoholic resorcinol method (7), or this method as modified by McRary and Slattery (4), may be used. The following values are determined directly: (*a*) total free sugars (from cold-water extract); (*b*) total free fructose (from cold-water extract); (*c*) free sugars plus levulins (from cold-water extract); (*d*) free sugars, levulins, and inulin (from hot-water extract); and (*e*) total fructose, free and polymerized (from both cold- and hot-water extracts, respectively). The required values not directly determined are obtained by subtraction: nonfructose free sugars, $a - b$; levulins, $c - a$; and inulin, $d - c$.

Fractionation of levulins

The basic values for free sugars plus 89%-ethanol-soluble levulins are determined as follows: 1- to 2-gm. samples of the finely ground tissue are continuously extracted with 89% ethanol, by volume, in a reflux apparatus, Landsiedl type, for 6 hours. However, the Soxhlet type extractor may be substituted if desired. For purposes of extraction, 80% ethanol, by volume, is placed in the boiling flask of the extractor, and thus the condensate dripping into the extraction cups will be approximately 89% ethanol by volume (11). The insoluble residue is discarded. The flask containing the ethanol extract is placed on the steam bath, and the ethanol is evaporated off. The 89%-ethanol-extract residue is transferred quantitatively to 100-ml. flasks and is made up to volume with water. Aliquots of this solution, hydrolyzed with HCl, neutralized with dilute NaOH, and clarified as indicated in the previous section, are used for the determination of free sugars plus 89%-ethanol-soluble levulins

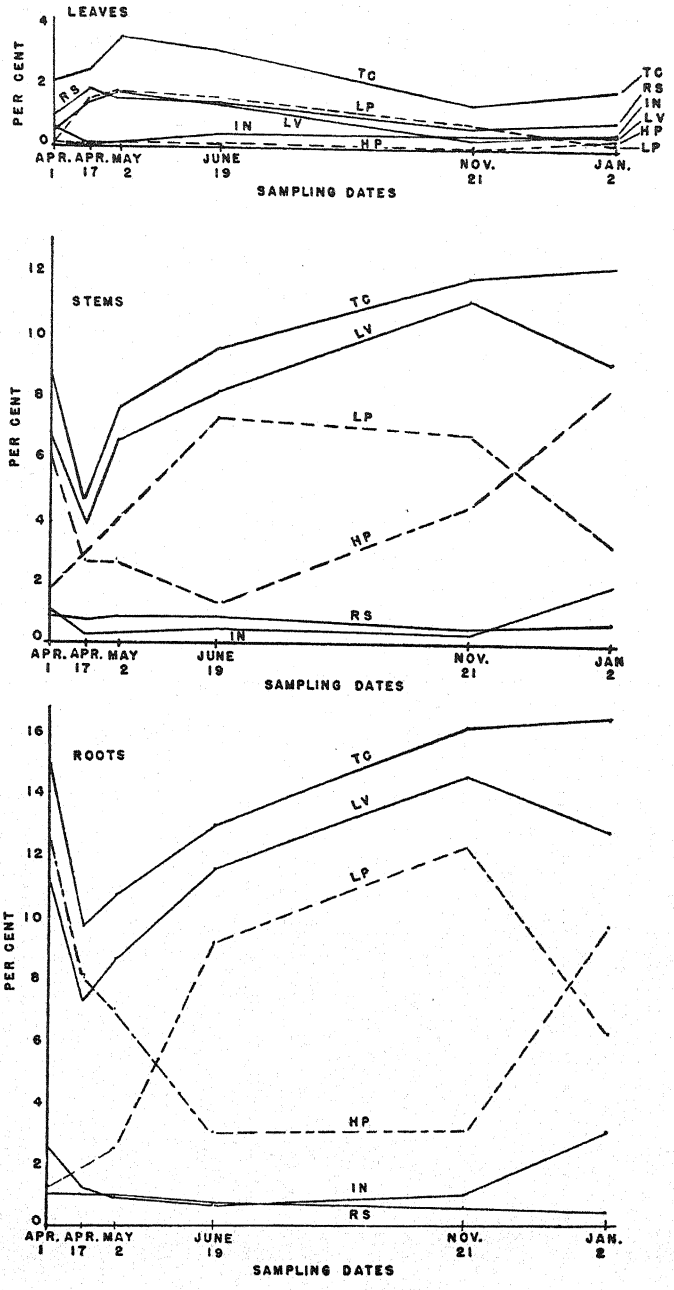


FIG. 1.—Seasonal changes in 2-year-old guayule nursery plants—leaves, stems, and roots; free sugars (*RS*); levulins (*LV*); inulin (*IN*); 89%-ethanol-soluble levulins (*LP*); 89%-ethanol-insoluble levulins (*HP*); and total water-soluble carbohydrates (*TC*); expressed on dry-weight basis.

by SOMOGYI's semimicro copper method (8) as modified by BROWNE and ZERBAN (1).

The values for the 89%-ethanol-soluble levulins are obtained by subtracting the values for free sugars from the values for free sugars plus 89%-ethanol-soluble levulins. The values for the 89%-ethanol-insoluble levulins are obtained by subtracting the values for free sugars plus 89%-ethanol-soluble levulins from the values for total water-soluble carbohydrates (free sugars, total levulins, and inulin).

Seasonal changes

Typical results obtained by the application of the methods briefly described above are shown graphically in figure 1. Seasonal changes in the various fractions of the water-soluble carbohydrates in guayule leaves, stems, and roots will be briefly discussed.

Free sugars (*RS*) are relatively limited in amount in the stems and roots, below 1%, but rise to almost 2% in the leaves. As contrasted with the relatively greater variation in levulins and inulin, there is little seasonal variation in percentage of free sugars in stems and roots, suggesting that the latter are the carbohydrates of translocation in guayule.

The inulin fraction (*IN*) is relatively small, never rising as high as 4%, in this case, in any of the tissues; it is largest in the roots, somewhat smaller in the stems, and very limited in the leaves. The inulin values rise with decrease in temperature in November, reach a peak in midwinter, and in April begin to fall again to the summer low.

Levulins (*LV*) are the chief reserve carbohydrates in the stems and roots of guayule at all seasons. The percentage of levulins is always highest in the roots, in which it falls to a minimum value soon after the first flush of growth in April. It rises rapidly until June and then gradually to the midwinter peak.

When the relative proportion of the 89%-ethanol-soluble levulins to the 89%-ethanol-insoluble levulins (11) is considered, we obtain a more dynamic picture of the carbohydrate transformations in guayule tissues. The values for 89%-ethanol-soluble levulins (*LP*) rise to a high during the summer and fall to a low in winter, beginning with the onset of lower temperatures, whereas the values for 89%-ethanol-insoluble levulins (*HP*) present a mirror image, falling to a low in summer and rising to a high in winter. TRAUB *et al.* (10) have shown that drought, or high soil-moisture, stresses, applied to guayule nursery seedlings, have an effect similar to that of low temperature.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
U.S. DEPARTMENT OF AGRICULTURE

LITERATURE CITED

1. BROWNE, C. A., and ZERBAN, F. W. Physical and chemical methods of sugar analysis. 3d ed. Wiley, New York. 1941.
2. HASSID, W. Z.; MCRARY, W. L.; DORE, W. H.; and MCREADY, R. M. Inulin in guayule, *Parthenium argentatum* A. Gray. Jour. Amer. Chem. Soc. 66:1970–1972. 1944.
3. JACKSON, R. F., and MATHEWS, J. A. Some physical properties of levulose and its estimation by copper reduction methods. U.S. Nat. Bur. Standards Jour. Res. 8:403–444. 1932. *Also in* BROWNE and ZERBAN. Physical and chemical methods of sugar analysis, pp. 824–825. 1941.
4. MCRARY, W. L., and SLATTERY, M. C. The colorimetric determination of fructosan in plant material. Jour. Biol. Chem. 157:161–167. 1945.
5. MCRARY, W. L., and TRAUB, H. P. Fructosan, a reserve carbohydrate in guayule, *Parthenium argentatum* Gray. Science 99:435–436. 1944.

6. NYNS, L. Selective determination of levulose by means of Ost's cupro-carbonate solution. Chem. Abst. 19:1236. 1925.
7. ROE, J. H. A colorimetric method for the determination of fructose in blood and urine. Jour. Biol. Chem. 107:15–22. 1934.
8. SOMOGYI, M. Notes on sugar determination. Jour. Biol. Chem. 70:599–612. 1926.
9. TRAUB, H. P. Rapid photometric methods for determining rubber and resins in guayule tissue and rubber in crude-rubber products. U.S.D.A. Tech. Bull. 920. 1946.
10. TRAUB, H. P.; SLATTERY, M. C.; and MCRARY, W. L. Effect of moisture stress on nursery-grown guayule with special reference to changes in reserve carbohydrates. Amer. Jour. Bot. 33: 699-705. 1946.
11. TRAUB, H. P., and SLATTERY, M. C. Levulins and inulin in guayule, *Parthenium argentatum* A. Gray. Plant Physiol. (In press.)
12. TRAUB, H. P.; SLATTERY, M. C.; and WALTER, E. D. Fructose and other monosaccharides in guayule, *Parthenium argentatum* A. Gray. Proc. Amer. Soc. Hort. Sci. 48. 1946.
13. TRAUB, H. P.; THOR, C. J.; WILLAMAN, J. J.; and OLIVER, R. Storage of truck crops: the girasole, *Helianthus tuberosus*. Plant Physiol. 4:123–134. 1929.

CURRENT LITERATURE

Die Koniferen des Oberkarbons und des unteren Perms. By RUDOLF FLORIN. *Palaeontographica* **85**, Abt. B (Hefte 1–7): 1–654. 1938–1944. Stuttgart. With pls. 1–186.

For some time, with interruption caused by World War II, Dr. RUDOLF FLORIN has been publishing in *Palaeontographica* his series of researches on Paleozoic conifers. These have appeared: Part 1 in 1938, Parts 2–4 in 1939, and Part 5 in 1940. Nearly all these earlier issues have reached American libraries. The printing of Parts 6–8 was delayed; Parts 6 and 7 bear 1944 dates and have thus far appeared in very few American libraries, while Part 8, which includes the summary, the all-important bibliography for Parts 6–8, and the index for the entire series, is in type but still awaits printing.

FLORIN has carefully examined at first hand fossil materials of the Upper Carboniferous, Permian, and Mesozoic. He gives detailed descriptions of hundreds of items found in European and American collections, all fully illustrated in 150 quarto plates (Parts 1–4). Here FLORIN applies his detailed knowledge of stomatal and cuticular anatomy founded upon his well-known researches[1] on these structures in the living conifers.

The fossil material of Paleozoic conifers is very abundant, but nearly all of it is in the form of impressions and compressions. Only the latter include some organic residues. True petrifactions are not available. Specimens of petrified Paleozoic wood are only doubtfully coniferous. In spite of the unsatisfactory condition of these fossils, it was possible to bring together the specimens belonging to many species under various genera through comparison of their cuticular characters and to recognize the reproductive parts that belong to the same system.

FLORIN has segregated from the fossil material, which had been referred to *Walchia* Sternb. more than a century ago, two Upper Carboniferous and Lower Permian genera: *Lebachia* Florin (Parts 1–3), under which he recognizes fourteen species, and *Ernestiodendron* Florin (Part 4), for which he describes several species. Others are left, and several new species are placed, under *Walchia* and related either to *Lebachia* or *Ernestiodendron*.

Part 5 includes about fifteen form genera, many of them based upon reproductive parts. These have all been subjected to the same detailed treatment and are illustrated in plates 151–166. Also included in Part 5 are keys to these fossil genera and a bibliography of 282 titles specifically for Parts 1–5.

Parts 6 and 7, dealing in summary with the vegetative and reproductive organs of fossil and modern conifers, are most interesting to the reviewer. These chapters include more general discussions, reconstructions, and comparisons. In addition to plates 167–186, there are 65 text figures, many of them in full page, showing FLORIN'S restorations of the Paleozoic and Mesozoic conifers.

The Lebachias were monoecious plants of moderate size, having small leaves always spirally arranged, small cones, and small seeds. Their growth habit resembled that of *Araucaria excelsa*. The leaves were small, closely imbricated, awl-shaped, slightly dimorphic, and with a single mid-vein. Those clothing the main stem and leaders were slightly larger than those on smaller secondary branches and had the apex forked, as in leaves of *Gomphostrobus*. Smaller leaves had acute, unforked apices.

The seed cones and pollen cones of *Lebachia* were

[1] R. FLORIN, Untersuchungen zur Stammesgeschichte der Coniferales und Cordaitales. I. Morphologie und Epidermisstruktur der Assimilationsorgane bei den rezenten Koniferen. K. Svenska Vet.-Akad. Handl. 3. ser. Vol. 10. Stockholm. 1931.

terminal on short lateral branches and always monosporangiate. No evidence was uncovered to indicate bisporangiate cones. The pollen cones were simple strobili with narrowly triangular, peltate, acute sporophylls spirally imbricated on the cone axis. Two microsporangia were attached beneath the sporophyll shield on opposite sides of the stalk. The pollen grains were peculiarly winged all around, with the exoexine almost wholly surrounding the included cell.

The seed cones were compound structures having the reproductive parts borne on short shoots or fascicled in the axils of bracts. The subtending bracts were forked at the tips after the pattern of the *Gomphostrobus* leaf type. In the axils of these bracts occurred free sterile and fertile stalks (interpreted as telomes by the author) intermingled. The seeds were carried singly and erect at the tips of the fertile appendages. *Ernestiodendron* was very similar to *Lebachia*, but some of the other genera of the period (various *Walchia* species) differed in having the seeds inverted at the ends of the stalks.

Part 7 includes descriptions of the reproductive parts of Cordaitales of two principal types. This is followed by a chapter of comparison of the Paleozoic conifers with the Cordaitales. Also discussed are the Upper Permian conifers, *Pseudovoltzia* Florin and *Ullmannia* Goeppert. As FLORIN reconstructs the cone-scale complex of *Pseudovoltzia*, it is composed of nine members unfused, except possibly at the base: three inverted ovules are borne at the ends of as many long stalks, behind which are five sterile leaflike members, and the lanceolate bract which subtends this assembly. It is through fusion of these members with each other and finally with the bract that the cone scale of the Pinaceae, Araucariaceae, Taxodiaceae, and other conifers has been derived.

FLORIN also considers a selection of generic types from the Mesozoic, with his own diagrammatic reconstructions and interpretations. This discussion considers about twenty Mesozoic conifers, with the Taxales set up as a separate order. The living conifers (with fifty genera) are then interpreted in the light of the fossil evidence presented. They are described under Taxodiaceae, Pinaceae, Araucariaceae, Podocarpaceae, Cupressaceae, and Cephalotaxaceae. The four genera of Taxaceae—*Taxus*, *Austrotaxus*, *Torreya*, and *Amentotaxus*—are here included under the Taxales, as an order distinct from the Coniferales. He finds *Callitropsis* Compton (not Oersted) a later homonym and supplies the new name *Neocallitropsis*.

There can be no question about the immense value of the study of cuticular anatomy to the identity of the conifers, other gymnosperms, and pteridophytes. Through this approach, FLORIN has been able to recognize and define fossil genera, including the least-known conifers of the Paleozoic. These new monographic studies will be indispensable in a study of conifers from all geological horizons. His cuticular investigations must be considered in any future monographic treatment, including that of living conifers.

From FLORIN's evidence, it appears that the old controversy concerning the nature of the coniferous cone scale has been definitely settled on the basis of historical data. The ovuliferous scale is an axillary structure or brachyblast, entirely separate from the bract in the compound seed cone of these fossils. The ovules, however, are megasporangia borne on their own stalks, not on the surface of the ovuliferous scale. In the course of evolution, these stalks fused with one another, with associated sterile stalks, and eventually with the subtending bract. To speak of the ovuliferous scale as a ligular outgrowth or appendage of the bract or as a "carpel" is obviously erroneous.

The reviewer is not fully convinced that the separation of the Taxales in a separate order is necessary. True, these are the "conifers without cones," meaning seed cones, but they all possess pollen cones and are a part of the conifers. This emphasizes the fact that the Taxales go far back in their history to the early Mesozoic. FLORIN considers that in the Taxales the female reproductive organs are not reduced from complex cones to single ovules but rather that this group never possessed seed cones and that the erect ovule was held over or derived somewhat directly from the erect ovules and seeds found in the Lebachias and earliest conifers. This condition held over, and it is still to be met with in *Voltziopsis* of the Mesozoic.

FLORIN's work in its entirety is requisite for any study of fossil plants. It is equally indispensable as a background to workers in the field of living conifers. —J. T. BUCHHOLZ.

GROWTH-REGULATING SUBSTANCES AS HERBICIDES

E. J. KRAUS AND J. W. MITCHELL[1]

Introduction

Soon after the discovery of the possible use of indoleacetic acid as a means of inducing root formation by some plants, it became obvious that there was a close relationship between the quantity and concentration of the acid used and the degree and type of response made by treated plants. It was clear, also, that the period of time over which a plant was exposed to the growth-regulator was important. It was necessary, therefore, to observe a critical quantity–concentration-time factor in the treatment of cuttings, seeds, or entire plants for any desired result (4, 10, 16, 17). Other significant observations were that various species, varieties, and even individual plants showed a marked differential in the degree of their responses to different substances in varying concentration under the same environment and that the responses were markedly influenced by varying environmental conditions. It was found that the chemical composition and the degree of maturity of the plant treated had much to do with its qualitative and quantitative responses after treatment. As more potent growth-regulators were discovered and studied, and as attempts were made to adapt them to economic use, the importance of all these facts became more strikingly clear and obvious. Many of the earlier, and also of the current, investigations have been concerned with the testing of plant behavior in these respects. Only a few of many earlier references are given to indicate these points (10, 11, 12).

Briefly summarized, it had been recognized almost from the inception of the attempted economic use of synthetic plant hormones or growth-regulating substances that there was a fairly wide range in the degree to which various species or even individual plants would respond. It was necessary to determine experimentally the range within which these compounds could be applied to plants without toxic injury (4, 10, 17). For many years, in the United States at least, although apparently less so in Great Britain (14, 17), few attempts were made to utilize growth-regulators as plant toxicants. Much greater attention was given to the potential capacity of growth-regulators to suppress bud development or to stimulate or increase plant growth, and other miscellaneous effects, especially by experimentalists in the applied fields. That there has been a marked change in viewpoint is well shown by the very large numbers of recent reports on the use of growth-regulators as herbicides. It is greatly to be hoped that a reasonable balance in viewpoint will prevail and that studies on the full range of the effects which these compounds may produce in and upon plants will receive the adequate attention such studies well merit.

When, prior to 1941, items of national defense became of increasingly greater concern, it was obvious to us, because of the closeness with which we had worked on growth-regulating substances for several years, that far more thought

[1] Senior Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md.

and attention should be given to any defensive measures which could be employed if advantage were to be taken of the toxic properties of growth-regulating substances for the destruction of crops or the limitation of crop production. Obviously, such studies could, at the same time, demonstrate any value which such compounds might possess as offensive tools. Special advantage could be taken of the differential sensitivity of plants to growth-regulators by using the latter as differential herbicides and in other ways in agricultural practice. What we suggested was that the experience and knowledge already at hand, together with that from additional investigations concerning plant responses to growth-regulators, be used more nearly completely than had been the case. Our suggestions were brought to the attention of certain groups of individuals which had been organized to concern themselves with problems of national defense. The idea was also presented to representatives of the United States Army. First at the University of Chicago and then under sponsorship of a committee of the National Academy of Sciences, investigations were begun and results reported from time to time. The work eventually became a part of an established program on biological warfare. All details concerning this work, as well as all results obtained, were promptly placed under restriction. Some of the pertinent facts concerning the program on biological warfare and its implications were presented by Mr. G. W. MERCK (3, 9) at the Westinghouse Centennial Forum in May, 1946. These need not be reviewed here. Some of the results of work carried out as part of the activities of investigators in the Special Projects Division, Chemical Warfare Service, at Camp Detrick, Frederick, Maryland, have appeared in a series of eighteen papers in the BOTANICAL GAZETTE for June, 1946 (107:476–632), and elsewhere (2). This program dealing with the use of growth-regulators as herbicides was well advanced before it was known to us that important developments in a similar field also took place in Great Britain (15, 18).

As has been pointed out by NORMAN (13), our earlier data have been withheld from publication up to now in conformity with security regulations. It still seems worth while to release them because they present certain results concerning the reactions of a number of species and varieties of plants apparently not yet recorded elsewhere. The points concerning the recovery and subsequent behavior of plants injured in varying degrees by the several growth-regulators, the resultant, obvious stimulating effects, as indicated by the increased intensity of the green color, larger size, and greater longevity of some plants, as well as various other details, deserve much further investigation.

In our earlier experiments growth-regulating substances then in common use were applied as sprays, dusts, and vapors and by other methods. By request, experiments with some of the better-known inorganic and organic herbicides, both differential and nondifferential, were carried out and the results reported. Among these materials three plant growth-regulators appeared to have some importance. In the case of bean plants, phenylacetic acid applied at the rate of 1 p.p.m. in water to soil decreased growth 75%. As an emulsion sprayed on the foliage, it decreased growth 10% compared with controls. Naphthaleneacetamide applied at the rate of 1 part in 10 million in water to soil decreased growth 70–100%, and the same concentration applied as an emul-

sion spray to the foliage reduced growth 25%. These results were somewhat similar to those already reported by other investigators (5, 6, 8, 14, 17). A 20% emulsion spray of α-naphthaleneacetic acid killed 70% of the treated plants. Results with other compounds were similar to those already frequently reported in the literature as dwarfing growth and, generally, decreasing yields of fruits or grains (10, 14, 17).

rophenoxyacetic acid, and 2,3,5-triiodobenzoic acid (fig. 1). A trial with most encouraging results was made in January, 1943, in the greenhouses at the University of Chicago. More extensive investigations were begun as soon as possible at the United States Plant Industry Station, Beltsville, Maryland. Data on some of the earlier trials are given herewith. In the descriptions below, trials are distinguished by letter for convenience in reading; the letters do not indicate the number or the sequence of the experiments as conducted.



FIG. 1.—Black Valentine bean plants. *Left*, untreated. *Right*, sprayed with a water emulsion containing 0.5% of 2,3,5-triiodobenzoic acid and 1% of Emulphor AG; 3 weeks after treatment.

Early in 1942 ZIMMERMAN (1, 19, 20) called attention to the high potency of certain substituted phenoxyacetic acid and benzoic acid growth-regulators. It occurred to us that these might well be of great value in connection with the experiments we had under way. Accordingly, samples of some of these materials were requested from Dr. ZIMMERMAN, who promptly furnished us with material of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 4-chlo-

Experimentation

TRIAL A

Young kidney bean plants were grown in rich soil in pots placed on well-lighted greenhouse benches at the Plant Industry Station. Two plants were left in each pot. At the time of treatment the first

trifoliate leaf was just beginning to expand. The plants were decapitated just below the node at which such a leaf was borne. Each growth-regulator used was added to lanolin, making a 2% mixture, and was applied in a thin film to the cut surface on March 22, 1943. The following notes were made on March 30, 1943.

Tryptophane.—Growth about same as in previous experiments (7); somewhat less than when glutathione was added to mixture.

Tryptophane (2%)+glutathione (2%).—Considerable growth made by entire plant; more than controls. Tumors formed show more growth than controls. Tumors irregular, large; many large galls formed at points where shoots had been removed. Galls about same size as tryptophane only but somewhat fewer in number. May be no correlation with composition of mixture applied.

α-Naphthyl methyl acetate.—Plants very responsive; tissues killed at surface and much swollen below. Many root primordia. Tumors decidedly ridged in appearance; resemble those from application of naphthaleneacetic acid more than any others.

4-Chlorophenoxyacetic acid.—Stem dead to primary leaves, which have turned yellow. Some injury below primary leaves. A few plants show tumors with dead stem above them. Mixture too concentrated.

2,4,5-Trichlorophenoxyacetic acid.—Results similar to application of 4-chlorophenoxyacetic acid. More plants show severe injury, especially farther down the stem. Leaves yellowed; some plants dead.

2,4-Dichlorophenoxyacetic acid.—Less injury than 4-chlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid. Tumors flatter when formed—more thickening of stems at greater distance. Substance apparently travels farther through plant, but plants are too severely injured to continue long development. Try weaker mixture.

α-Naphthaleneacetic acid.—Large tumors as usual, about like those resulting from application of α-naphthyl methyl acetate but appreciably smaller; not so flat; fewer roots, about same amount of injury.

Trial B

Another set of lanolin mixtures was applied on April 9, 1943, at Beltsville. The bean plants were grown in pots of rich loam in a well-lighted greenhouse, two plants per pot. They were more vigorous than the first lot treated on March 22, 1943. The decapitation method was used; the trifoliate leaf was expanding at the time of treatment.

Notes were made and specimens collected on April 26, 1943. The concentration of each growth substance used is given for each particular treatment.

Tryptophane (2%).—From the same bottle used in trial A. Results about the same as in first experiment, but many fewer galls developed where shoots had been removed. Since there was more space between plants, there may have been less moisture in the air around the plants. Plants grew more rapidly than in trial A, but tumors were no larger.

Tryptophane (2%)+glutathione (2%).—This mixture was the same as used in trial A, and results are similar. Addition of glutathione to the tryptophane mixture gives definite stimulation in early stages of growth but does not result in a finally larger tumor. Tumors on sides of stems somewhat larger than with tryptophane only. This may be the result of more light and a difference in moisture content of the atmosphere.

4-Chlorophenoxyacetic acid (1%).—A thin film was applied on cut surface, but some ran down the side when it melted in sun. Material from same vial as in trial A, but diluted with an equal volume of lanolin. Treated stems usually killed halfway to primary leaves, where they then enlarged to form large oval tumors with prominent ridges and some root primordia. Axillary shoots 1–5 inches long; some normal, but most of them have tightly rolled, very narrow leaflets, curled at the tip. Leaves striped dark- and light-green like those on plants showing symptoms of mosaic. Stimulus apparently travels for long distances in the plants. Primary leaves yellow. Axillary stems not much distorted. (Fig. 2*B*, *D*, *E*.)

2,4-Dichlorophenoxyacetic acid (1%).—Results similar to applications of 4-chlorophenoxyacetic acid. Tumors flatter; more root primordia, about 2 mm. below cut surface. Centers of tumors dead. Lateral shoots more deformed than with 4-chlorophenoxyacetic acid, often thickened and incurved like crab claws. This effect is much more pronounced than that fol-

lowing application of any other chlorophenoxyacetic acid compound. Some shoots are nearly normal if tumor is small, but many show some of the upper leaves with mosaic-like symptoms, the green areas greener than normal. This compound translocated for the longest distances of any tried and apparently is most toxic of any applied. (Fig. 2*A*.)

2,4,5-Trichlorophenoxyacetic acid (1%).—Growth responses similar to the two former chlorophenoxyacetic acid compounds, but tumors larger and flatter, flanged, the flanges with roots. Center of tumors dead. There are some root primordia on the stem above primary leaves, but tumor is distinctive. Some axillary shoots appear nearly normal and have a few flower buds; some have strong mosaic-like appearance; a few with crab claws at tips of shoots. Apparently translocated less distance and has less destructive action than 4-chlorophenoxyacetic acid or 2,4-dichlorophenoxyacetic acid. (Fig. 2*C*.)

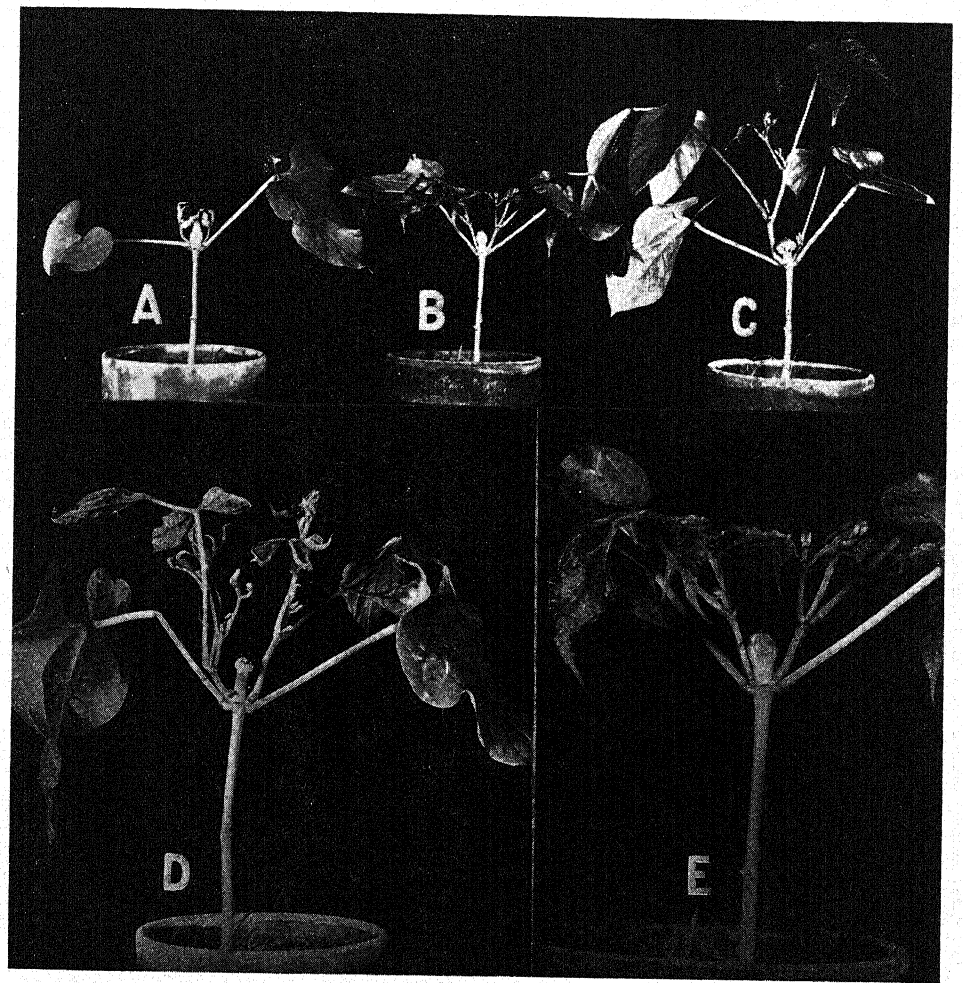


FIG. 2.—Response of Red Kidney beans to terminal applications of 1% mixtures of three different chlorophenoxyacetic acids in lanolin. *A*, 2,4-dichlorophenoxyacetic acid; *B*, 4-chlorophenoxyacetic acid; *C*, 2,4,5-trichlorophenoxyacetic acid, showing large flanged tumors; *D*, 4-chlorophenoxyacetic acid; *E*, closer view of *B*. *B*, *D*, and *E* show what is referred to in text as effects resembling "mosaic-like symptoms." Treated April 9, 1943; photographed April 26, 1943.

TRIAL C

A third set of mixtures was applied in the greenhouses at Beltsville. As in the other trials, the beans were thinned to two per pot. The plants were more vigorous and larger than in either of the other two trials. The trifoliate leaves were just spreading out; on a few plants they still were not folded down. The weather was warmer, and there was more sun than for previous lots of plants. Fewer of the plants were slender. The decapitation method was used, and lanolin mixtures at the concentrations indicated were applied to the cut surfaces on May 11, 1943. The following notes were made on May 26, 1943.

N-(β-naphthyl)-glycine (1.5%).—Very slight response; no killing; large axillary shoots apparently not injured—many with large buds and few flowers. About like lanolin-treated controls.

Benzothiazyl thioglycolic acid (1.5%).—Very little response, more than to foregoing but not so much as made by some of controls, which have large phloem galls. Shoots not injured; buds and some flowers about as in controls.

β-Naphthoxy α-propionic acid (1.5%).—Extremely large, knobby tumors dead at center, flanged, and with many root primordia. A few plants grew very little. Not much apparent effect below tumors. Shoots large, similar to controls. A few petioles and blades of the primary leaves have tumors with roots. Translocation questionable; tumors on leaves possibly resulted from contact with tip of stem while being treated.

α-Naphthyl methyl acetate (1.5%).—Extremely large tumors, biggest of any in this experiment. They resemble those resulting from application of β-naphthoxy α-propionic acid as indicated above but are larger, less tuberculated, and with fewer roots. Some flanges on stem below tumor; a few root primordia $\frac{3}{4}$ inch from treated surface. Axillary shoots dwarfed somewhat (often only one large one), shorter than controls. Plants smaller but about same stage of bloom as controls.

Phenazine (1.5%).—Little or no tumor response, but tissues apparently killed near surface. No tumors, as is true for controls. Axillary shoots about same as those of controls. Flower buds and few flowers present.

Of the same lot of plants as those used for the terminal applications to decapitated internodes, some were thinned to two plants per pot and arranged in rows on the greenhouse benches. A thin thread of lanolin–growth-substance mixture was wound around the second internode just below the trifoliate leaf. The same mixtures and concentrations as were used in making the terminal applications were employed. Growth substances were applied on May 11, and notes were taken on May 25, 1943.

N-(β-naphthyl)-glycine (1.5%).—Stems yellowed, some showing swelling although scarcely visible. No apparent injury or stimulation.

Benzothiazyl thioglycolic acid (1.5%).—Apparently no response other than yellowing of stems at point of application and slight spindle-like swelling. Plants resemble controls in all respects.

β-Naphthoxy α-propionic acid (1.5%).—Many large spindle-shaped tumors, $\frac{1}{4}$–1 inch long, flanged with many root primordia. Some tips died soon after treatment. Such plants are sometimes dead to the primary leaves. Often they have big terminal tumors resembling those following terminal applications, but with dead stem tip at center. Slight dwarfing effect; otherwise like controls.

α-Naphthyl methyl acetate (1.5%).—Very great response, long spindle-shaped tumors, sometimes nearly full length of an internode. Larger portion of tumor is below line of treatment than above it. Some terminals dead, others vigorous, especially if only small tumor is present. Tumors rough, flanged, many root primordia and rather spongy. Little effect on the primary leaves (many are shed) or on portion of plant below them. If big tumor is present and stem tip is dead, strong axillary shoots are present similar to controls. Response somewhat resembles that to naphthaleneacetic acid except it is greater.

Phenazine (1.5%).—No visible effect.

4-Chlorophenoxyacetic acid (1%).—Very large long galls, sometimes extending below primary leaves. Tips of stems frequently killed or much swollen, resembling small potatoes above trifoliate leaf which often shows effects resem-

bling symptoms of mosaic. Axillary shoots much as in terminal treatment except that mosaic-like appearance is more pronounced and leaves are curled, especially smaller leaves, which often are yellow.

2,4,5-Trichlorophenoxyacetic acid (*1%*).—Very similar to responses to 4-chlorophenoxyacetic acid except more pronounced; effects show far greater distance down the stem. These effects seem greater than following terminal application of mixture. Some shoots have flower buds and flowers. Leaves are large as in controls; green areas of leaves which show mosaic-like symptoms seem darker. Some of the "unaffected" leaves also appear darker than those of controls. Whole plant is more bushy than controls.

2,4-Dichlorophenoxyacetic acid (*1%*).—In nearly every respect similar to responses following application of 2,4,5-trichlorophenoxyacetic acid, but plants seem more branched and bushy. They are much dwarfed compared with controls. Some have died completely, starting from the tops down. Earlier they showed root primordia and swelling below primary leaves. On some nearly dead plants, a leaf or two still appears fairly green or partially yellow. Some plants have many small shoots at cotyledonary nodes. These shoots have many leaves and clusters of buds. Most of the live plants are twiggy in appearance; others have large green shoots. Responses very variable. This may be the result of the degree of contact made in application, or owing to individual response of plant treated.

Controls (*May 26, 1943*).—Plants which had been decapitated are now 16–20 inches tall. Many of them have flower buds, flowers, and young fruits. Many of the shoots in the axils of primary leaves are as large as is the main axis below them. The primary leaves of many plants are yellowed or abscised. Plants which were treated laterally with lanolin only are generally similar to plants treated terminally with lanolin only, except that they are somewhat taller.

Trial D

During the period in which some of the foregoing experiments were in progress at Beltsville an experiment dealing with the spraying of rice was begun and carried out at the University of Chicago. Two varieties of rice, Blue Rose and Fortuna, were grown in large concrete tanks in a greenhouse. On May 14, 1943, the plants were about 12–14 inches tall, vigorous, but not yet showing heads. The water in the tanks was generally maintained $1\frac{1}{2}$–2 inches in depth and developed a very slight surface scum. Three of the tanks were 84×36 inches; the fourth was 76×36 inches. The water level in the tanks was about $\frac{3}{4}$ inch deep when they were sprayed. One tank was sprayed with 100 mg. of 4-chlorophenoxyacetic acid, another with the same quantity of 2,4-dichlorophenoxyacetic acid, and a third with the same quantity of 2,4,5-trichlorophenoxyacetic acid. Before application each compound was first mixed with 5 gm. of melted lanolin and then made up to 100 cc. as an emulsion with a 5% solution of laundry soap.

These emulsions were prepared by dissolving the required amount of growth-regulator in melted lanolin and then pouring this melted mixture into the hot soap solution while being stirred vigorously in a Waring blendor. When well mixed, the emulsion was diluted to any desired volume. Because of the alkalinity of the soap used in preparing the emulsion, it is probable that salts of the several acids were formed. The diluted emulsion was then sprinkled over the surface of the water in the respective tanks. The emulsion spread slowly over the water, which became turbid. It was then stirred slightly with a stick to secure uniform spread of the emulsion over the surface and throughout the water. The material tended slightly to collect around the stems of the plants, forming a thin film on their surface, $\frac{1}{2}$ inch or more above the water level.

On May 16, 1943, it was noted that there was not much change in appearance of the treated plants except that all showed more yellowing of the tips of the

leaves than did the controls. No bending or other changes were observed except that the sheathing leaves at the base of the plants appeared slightly water-soaked somewhat higher above the water level than did those of the controls. These basal sheaths were dead and black in all plants. There was some apparent injury, but it was not nearly so pronounced as is usually shown by beans after the same length of time.

The next opportunity to make observations on these plants was on June 25, 1943. The unsprayed rice was green, and many of the plants showed the beginnings of heads or heads partially emerged. In the tank sprayed with 4-chlorophenoxyacetic acid emulsion, most of the rice plants had the leaves dead near the surface of the water. Some plants were fairly green but none had heads. Many were dead. Nearly all the plants sprayed with the 2,4,5-trichlorophenoxyacetic acid emulsion were dead and dried up except those near the corners of the tank. The appearance of the plants sprayed with the 2,4-dichlorophenoxyacetic emulsion was about the same as of those sprayed with 2,4,5-trichlorophenoxyacetic acid emulsion. This tank was in the northwest corner of the greenhouse and deeper than the others. The water in it might have been deeper when sprayed, and there was more distance from the water level to the top of the tank. 2,4-Dichlorophenoxyacetic acid was about as potent as 2,4,5-trichlorophenoxyacetic acid emulsion, but its application resulted in the killing of more plants than 4-chlorophenoxyacetic acid emulsion.

TRIAL E

Another trial was begun to test further the relative toxicity of certain substances and especially the degree to which they or their effects were translocated within the plant to which they had been applied. Red Kidney bean plants were used in the greenhouses at the University of Chicago. On September 26, 1943, when the substances were applied, the plants were vigorous but somewhat spindling because of the prevailing low light intensity. On most of the plants the first trifoliate leaves were expanding. On the others this leaf was fairly well expanded, and the internode above it was 2–3 inches long and rather slender. The plants were grown in pots of soil, two to each pot, and were spread out in rows on greenhouse benches. Each of the growth-regulating substances was dissolved in, or thoroughly mixed with, melted lanolin and applied at a 1.5% concentration. Applications were made in various ways: the usual terminal application after decapitation, thin bands applied laterally to uninjured stems, or in other ways as specifically indicated in each test. The appended notes were taken on October 8, 1943.

The chlorophenoxyacetic acid compounds used in this experiment were prepared by Dr. E. D. WITMAN, Ohio State University Research Foundation. Some of the other compounds used are of the same lot of materials mentioned in previous trials.

4-Chlorophenoxyacetic acid.—Terminal. Large tumors, many extending to soil line. Some plants have no lateral shoots; if present, these are small, deformed, and show mosaic-like symptoms. Some plants are dead and yellow.

Same.—Lateral. Long tumors; some terminating at node, others extend nearly to soil. Trifoliate leaves much distorted; tips of stems above leaves often thick, swollen, and yellow; some of them dead. Some whole plants dead with leaves yellow but not so many as in terminal treatment.

2,4-Dichlorophenoxyacetic acid.—Terminal. Very long, large tumors. Many plants killed, including roots; many show mosaic-like appear-

ance of lateral shoots similar to 4-chlorophenoxyacetic acid but more intense. Many roots on stems. Some plants very much thickened, yellow, dead or dying.

Same.—Lateral. Similar to terminal treatment. More plants dead. Tips of most plants above ring of treatment dead; some much curled, twisted, or tuber-like. Many tumors extend to soil surface; plants curled over. *Stronger killing action than 4-chlorophenoxyacetic acid.*

2,4,5-Trichlorophenoxyacetic acid.—Terminal. Very similar to 2,4-dichlorophenoxyacetic acid in all respects except that effects are less pronounced.

Same.—Lateral. Effects very similar to those of 2,4-dichlorophenoxyacetic acid applied laterally. Some plants seem to be more injured, others less so. Effects do not seem to extend so far throughout plant as in case of 2,4-dichlorophenoxyacetic acid.

2,4,6-Trichlorophenoxyacetic acid.—Terminal. Almost no tumor formed. Yellowing near point of application. Very slight growth at surface.

Same.—Lateral. Virtually no response. Very different from 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acid. (NOTE.—Repeat using new preparation or mixture.)

β-Naphthoxy α-propionic acid.—Terminal. Terminal tumors similar to those in previous experiments performed at Beltsville but not so large or extensive. Effects more localized. May be that plants are less active now; they are more spindling.

Same.—Lateral. Small spindle-like tumors very similar to those produced when naphthaleneacetic acid was applied, but somewhat more rough. Effects localized. Tops above treatment somewhat dwarfed, but no effects comparable in degree with chlorophenoxyacetic acid compounds.

α-Naphthyl methyl acetate.—Terminal. Medium-sized, spreading tumors recurved at edges; smaller than at Beltsville in spring, more localized, much less injury. Axillary shoots large.

Same.—Lateral. Similar to tumors produced by application of β-naphthoxy α-propionic acid but smaller and more localized. Not much injured, but plants are smaller than controls. Resembles effects of naphthaleneacetic acid, but tumors are somewhat smaller.

2,4-Dichlorophenoxyacetic acid.—Not decapitated. Applied as small pellet on vein at middle of primary leaf. Vein enlarged; yellow spot only on leaf, but effect very marked on axillary shoots and tips of plant which are either mosaic-like or thickened and swollen. Trifoliate leaves often curled, dwarfed, mosaic-like. Petioles of primary leaves show almost no swelling. Some leaves dead. Effects shown by axillary shoots are as great as when substance is applied terminally.

2,4,5-Trichlorophenoxyacetic acid.—Method of application same as above. Effects similar to those from 2,4-dichlorophenoxyacetic acid but average slightly less. Effects of conduction for long distances very marked. More mosaic-like in appearance with less swelling of tips of stems and young leaves than in case of 2,4-dichlorophenoxyacetic acid. (NOTE.—Repeat on younger and older plants.)

2,4,6-Trichlorophenoxyacetic acid.—Method of treatment same as for 2,4-dichlorophenoxyacetic acid above. Apparently no effect except small yellow spot. (NOTE.—See terminal and lateral applications. Repeat using new mixture.)

2,4-Dichlorophenoxyacetic acid.—Applied to tip of middle leaflet of partially expanded first trifoliate leaf above long, slender second internode. Leaflet sometimes killed. Much less effective than when applied on vein, but tip of stem above treated leaf often tuberous, sometimes dead. Other two leaflets of leaf often deformed. Sometimes no effect if tip of treated leaflet has died. Very little effect shown by axillary shoots.

2,4,5-Trichlorophenoxyacetic acid.—Applied as in 2,4-dichlorophenoxyacetic acid treatment. Effects similar to 2,4-dichlorophenoxyacetic acid. Tips of more leaflets killed; such plants often show no injury to tip of stem. Apparently less conduction following this method of application than when substance is applied on vein. Some leaves appear greener than those of controls.

2,4,6-Trichlorophenoxyacetic acid.—Method of treatment same. Plants are at east end of greenhouse and received more light. These plants are somewhat taller and more advanced than the others. No resultant effects after treatment except some tips of leaflets are yellow and a few dwarfed. Some of the primary leaves are yellow and have fallen. Plants resemble controls in all respects.

Indoleacetic acid.—Terminal. Tumors similar to those usually produced following application of this compound but are somewhat smaller than in some previous experiments.

α-Naphthaleneacetic acid.—Terminal. Responses similar to those usually following treatment with this compound, but tumors some-

what smaller and with fewer root primordia. Some tumors have died; in such cases the stem is dead to the primary leaves. Large axillary shoots.

Same.—Lateral. Similar to previous comparable experiments, except tumors are shorter and with not many tubercles or flanges. Tops above region of application dwarfed but not killed; few root primordia.

Same.—On midvein of primary leaf. Yellow spot on some leaves; others have raised area at point of application. Some leaves have medium-sized tumor with root primordia. Vein sometimes yellow. Leaf shows slight injury but may be strongly curled. Not much effect on axillary shoot. None of treated leaves abscised. They are greener in color than controls.

Controls (*lanolin only*).—Not decapitated. Many are vinelike, 24 inches tall, slender, with flower buds or flowers. More slender than plants which received more sunshine. Primary leaves yellowing, some fallen, others green. Plants at east end of greenhouse are larger than those near door at west end. Some of them with one or two flowers and some buds.

Same.—Decapitated. Large axillary shoots, a few with flower buds. Most of decapitated internodes shriveled to near primary leaves; a few have phloem tumors at their tips. Most of axillary shoots are not vinelike except some which are near door. These latter do not have so many buds as at east end of greenhouse where plants are more bushy.

NOTE.—Repeat experiments using chlorophenoxyacetic compounds. These are by far the most promising as herbicides and are conducted long distances in plants; other compounds result in more localized response. Try further emulsion sprays on leaves.

The lack of response to applications of 2,4,6-trichlorophenoxyacetic acid seemed surprising when compared with the other chlorophenoxyacetic acid compounds, but similar results were obtained with repeated trials. ZIMMERMAN and HITCHCOCK (**20**) have also reported like results.

TRIAL F

In January, 1944, another set of experiments was initiated at the University of Chicago.[2] Their immediate purpose was to determine, first, the most active of the compounds already tested and, second, to ascertain their effectiveness in decreasing the over-all growth and productivity of several types of plants when applied in relatively small amounts.

Barley, oats, corn, wheat, kidney bean, buckwheat, radish, rape, red clover, and sugar-beet plants were grown from seed in soil contained in flats which measured approximately 15×24 inches. In addition, two varieties of rice (Blue Rose and Fortuna) were grown in concrete tanks measuring approximately 36×72 inches and filled with soil to a depth of 6 inches. Seeds of the different plants were sown during January and early February. In the case of sugar beet and kidney bean two successive plantings were made so as to obtain plants of different ages. One sowing was made on January 16, 1944; the other, on February 3, 1944. The bean plants referred to as "old" in the photographs were sown on the former date; those as "young," on the latter. After the rice had germinated, a sufficient quantity of water was added to each tank to cover the soil to a depth of approximately 2 inches.

The plants were grown for a period of from 2 to 6 weeks before treatment, during which time relatively cloudy weather prevailed. At the time of treatment all plants, except early plantings of beets and beans, were yet immature and relatively succulent.

The growth-regulating compounds used included the most active of those known at the time these experiments were initiated, namely:

2,4-Dichlorophenoxyacetic acid
Penta-chlorophenoxyacetic acid

[2] Dr. CHARLES L. HAMNER, at the time Assistant Plant Physiologist in the Bureau of Plant Industry, Soils and Agricultural Engineering, assisted in the application of some of the compounds in this experiment and understood its objectives.

2-Chlorophenoxyacetic acid
4-Chlorophenoxyacetic acid
2,4,5-Trichlorophenoxyacetic acid
2,3,5-Triiodobenzoic acid
α-Naphthaleneacetic acid
α-Naphthyl methyl acetate

Data as to methods of treatment are given in table 1. The numbers used there are the same as those given in some of the data presented here and as shown on the small labels in the photographs (figs. 3–34). These labels also indicate the concentration of the growth-regulator used in each instance. All photographs were taken 10 days after treatment.

The 1% lanolin emulsions were prepared as follows. One gram of growth substance dissolved in 5 ml. of ethyl alcohol was added to 10 gm. of melted lanolin. This mixture was then poured into 100 ml. of hot 5% solution of laundry soap and thoroughly mixed in a Waring blendor. For the lower concentrations dilutions were made with a similar soap solution. Since the soap was strongly alkaline, it is probable that salts of the various growth substances were formed in the emulsions. This fact, and also the fact that there was little sunshine during the period of growth of the plants previous to treatment and for more than 10 days after treatment, may have resulted in less response on the part of some of the plants than might have been true in sunny weather. The emulsions were applied to the plants by means of hand sprayers at the rate of approximately 25 ml. per flat of plants (surface area approximately 2.5 sq. ft.).

Light- and heavy-oil emulsions were prepared in a manner similar to the lanolin emulsions except that 40 ml. (approximately 10 gm.) of Valvoline motor oil (S.A.E. 40 or S.A.E. 10) were substituted for the lanolin. The oil emulsions were applied in a manner similar to the lanolin emulsions.

TABLE 1

GROWTH SUBSTANCES USED, METHOD OF APPLICATION, AND CONCENTRATIONS USED, FEBRUARY 29, 1944

Trial number and substance used	Method of application	Concentration
1. 2,4-Dichlorophenoxyacetic acid	Aerosol	12.6% in oil
2. 4-Chlorophenoxyacetic acid	Aerosol	6.3% in oil
3. Penta-chlorophenoxyacetic acid	Aerosol	1.26% in oil
4. α-Naphthaleneacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
5. 2,3,5-Triiodobenzoic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
6. 2,4-Dichlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
7. 4-Chlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
8. 2-Chlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
9. 2,4,5-Trichlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
10. 2,4-Dichlorophenoxyacetic acid	Oil emulsion S.A.E. 40	1%, 0.5%, 0.25%, 0.13%
11. 2,4-Dichlorophenoxyacetic acid	Oil emulsion S.A.E. 10	1%, 0.5%, 0.25%, 0.13%
1. α-Naphthaleneacetic acid	Dust	1 part to 500 talc
2. α-Naphthaleneacetic acid	Dust	1 part to 5000 talc
3. α-Naphthyl methyl acetate	Dust	1 part to 500 talc
4. α-Naphthyl methyl acetate	Dust	1 part to 5000 talc
5. Penta-chlorophenoxyacetic acid	Dust	1 part to 500 talc
6. Penta-chlorophenoxyacetic acid	Dust	1 part to 5000 talc
7. 2,4-Dichlorophenoxyacetic acid	Dust	1 part to 500 talc
8. 2,4-Dichlorophenoxyacetic acid	Dust	1 part to 5000 talc

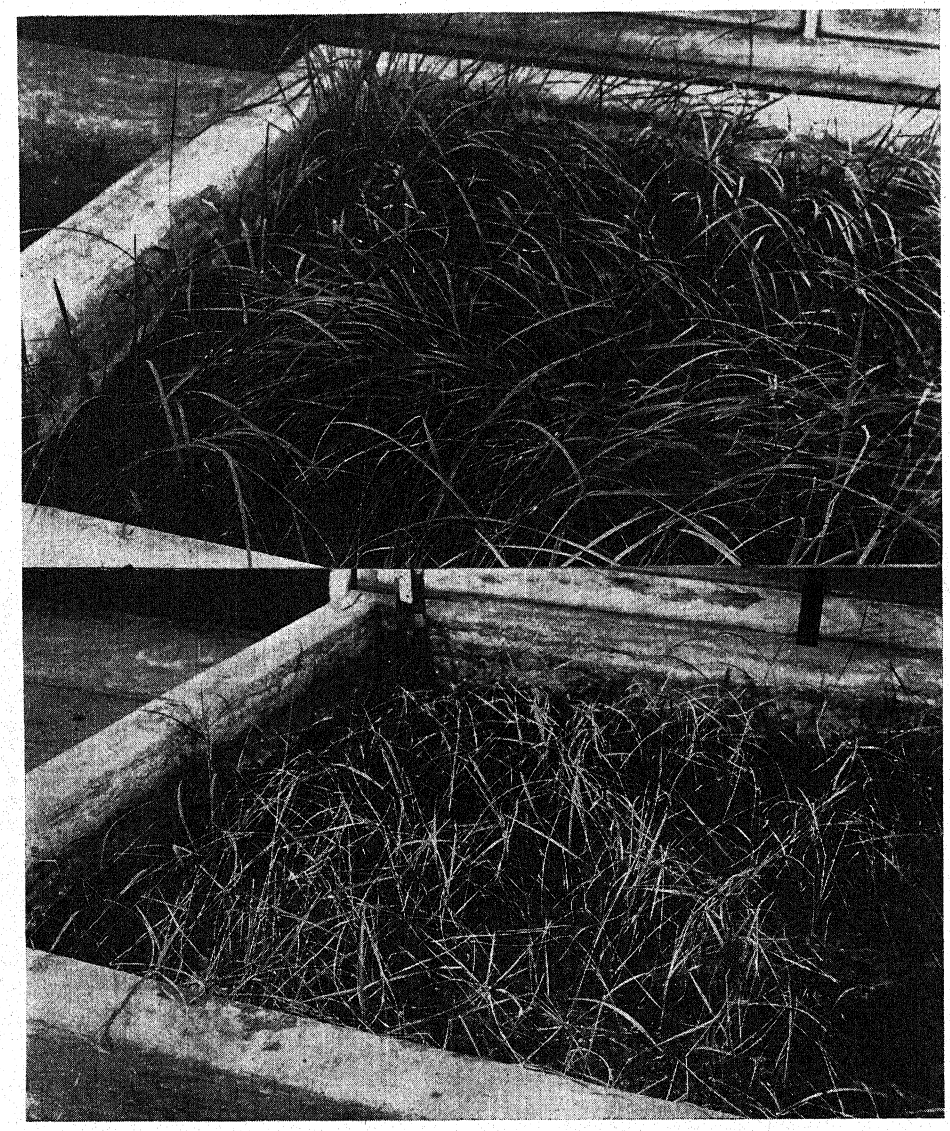


FIG. 3.—Blue Rose rice. *Above*, untreated plants 38 days after emergence. *Below*, comparable plants 10 days after spraying with an oil (S.A.E. 40) emulsion containing 1% of 2,4-dichlorophenoxyacetic acid, at the rate of 10 ml./sq. in. of water surface. Tank 36×72 inches. Water in tank ½ inch deep at time of spraying.



FIG. 4.—Barley 44 days old when treated. *Top*, untreated. *Center*, treated with aerosol containing 2,4-dichlorophenoxyacetic acid (*left*) and with 1% lanolin emulsion of the acid (*right*). *Bottom*, treated with 1% lanolin emulsions of 4-chlorophenoxyacetic acid (*left*) and 2,4,5-trichlorophenoxyacetic acid (*right*).



FIG. 5.—*Top*, wheat 44 days old when treated. *Left*, untreated. *Right*, treated with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, oat plants 44 days old when treated. *Left*, untreated. *Right*, sprayed with lanolin emulsion containing 2,4,5-trichlorophenoxyacetic acid.



FIG. 6.—Corn plants sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid (*top, left*) and with lanolin emulsions containing 2,3,5-triiodobenzoic acid (*top, right*), 2,4-dichlorophenoxyacetic acid (*bottom, left*), and 4-chlorophenoxyacetic acid (*bottom, right*).

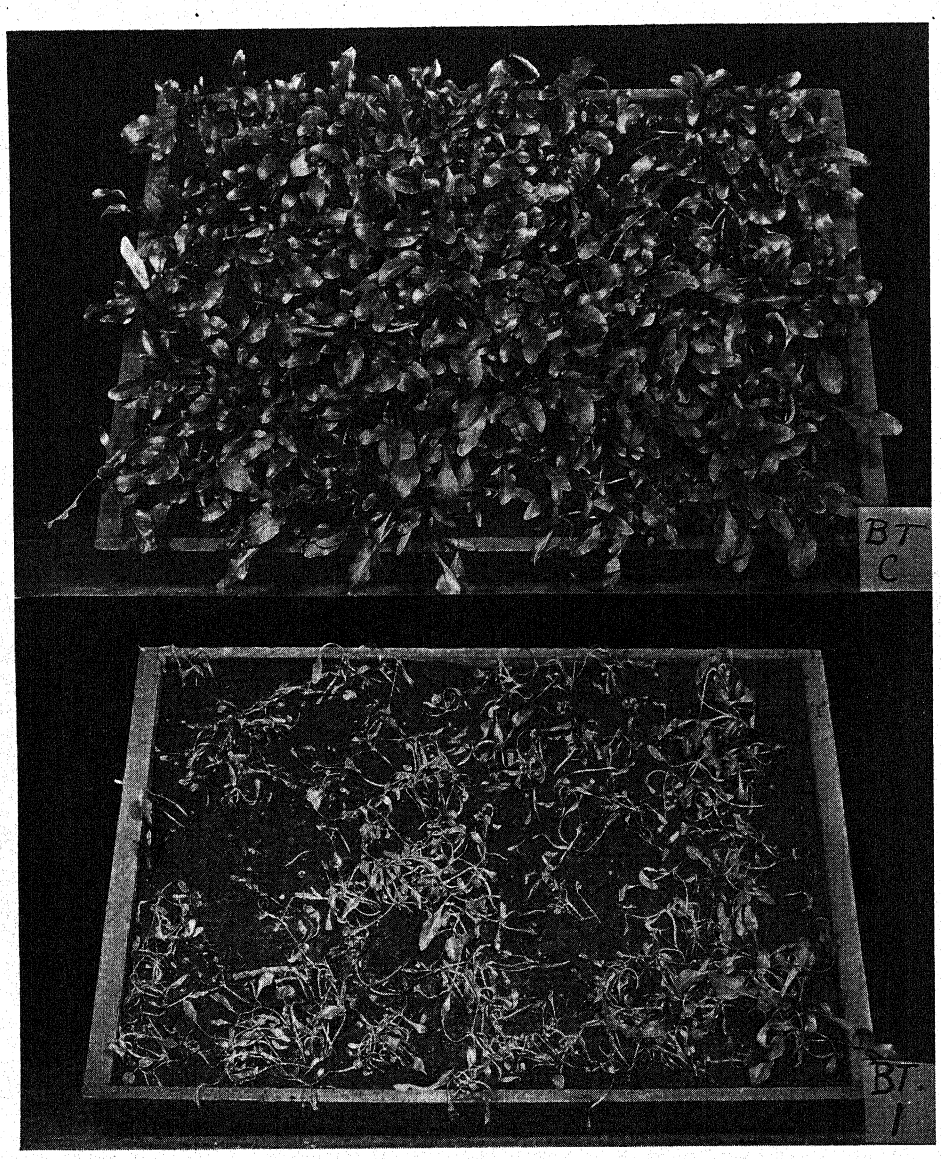


FIG. 7.—Sugar-beet plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with aerosol that contained 2,4-dichlorophenoxyacetic acid.



FIG. 8.—Sugar-beet plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*top, right*) of 2,4-dichlorophenoxyacetic acid and 1% (*bottom, left*) and 0.13% (*bottom, right*) of 2,4,5-trichlorophenoxyacetic acid.

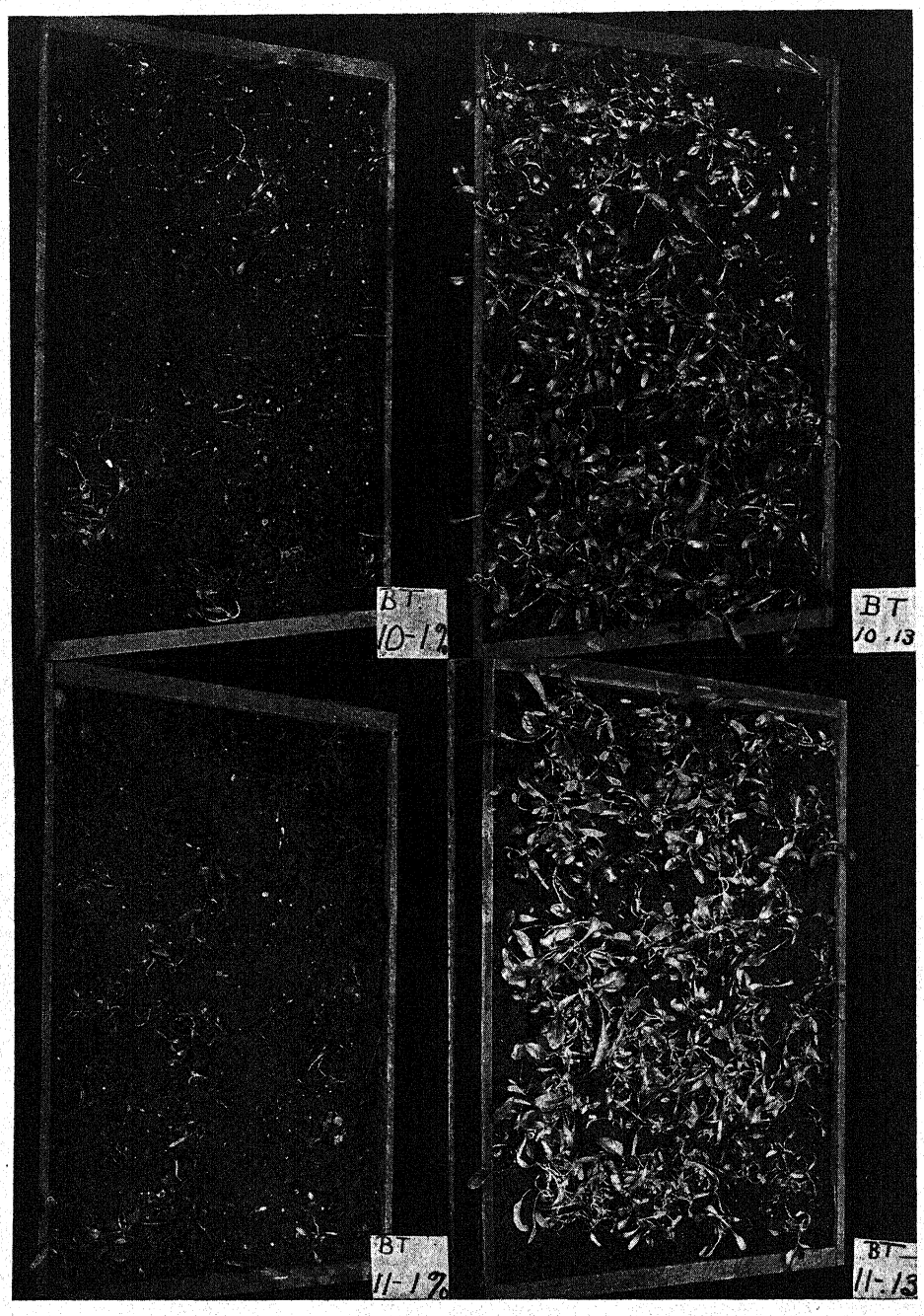


FIG. 9.—Sugar-beet plants sprayed with heavy-oil emulsions (*top*) containing 1% (*left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid and light-oil emulsions (*bottom*) containing 1% (*left*) and 0.13% (*right*) of the acid.

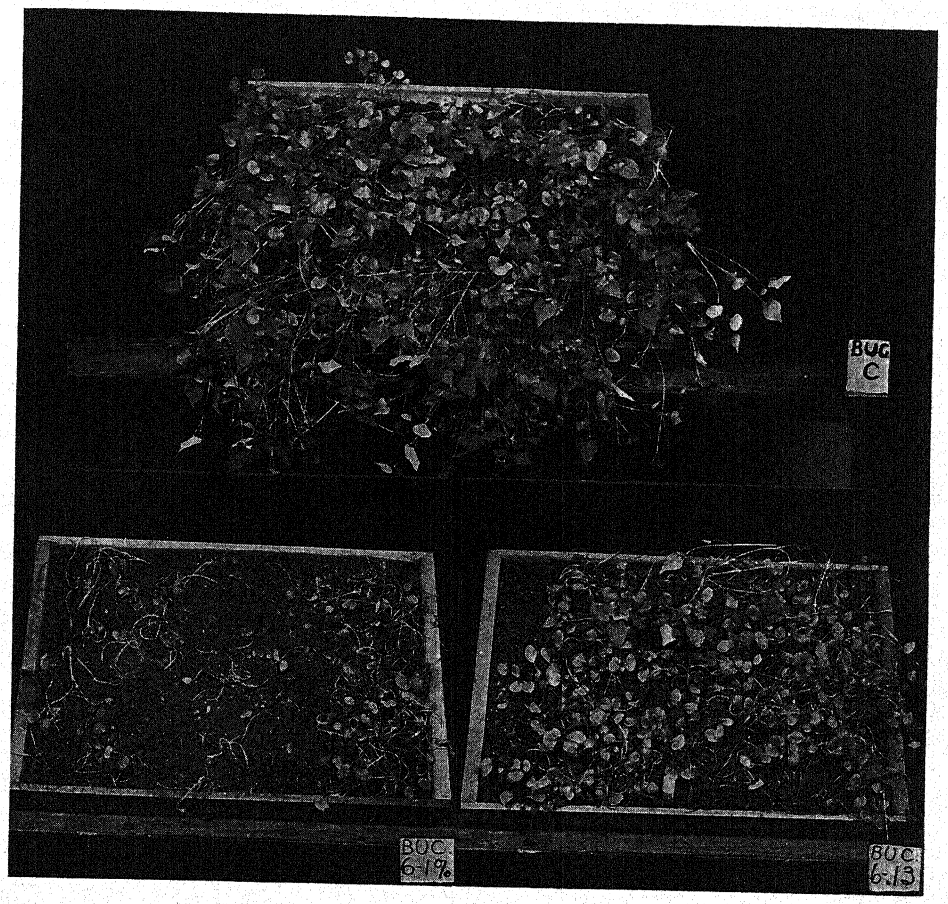


FIG. 10.—Buckwheat plants 26 days old when treated. *Top*, untreated. *Bottom*, sprayed with lanolin emulsions containing 1% (*left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid.

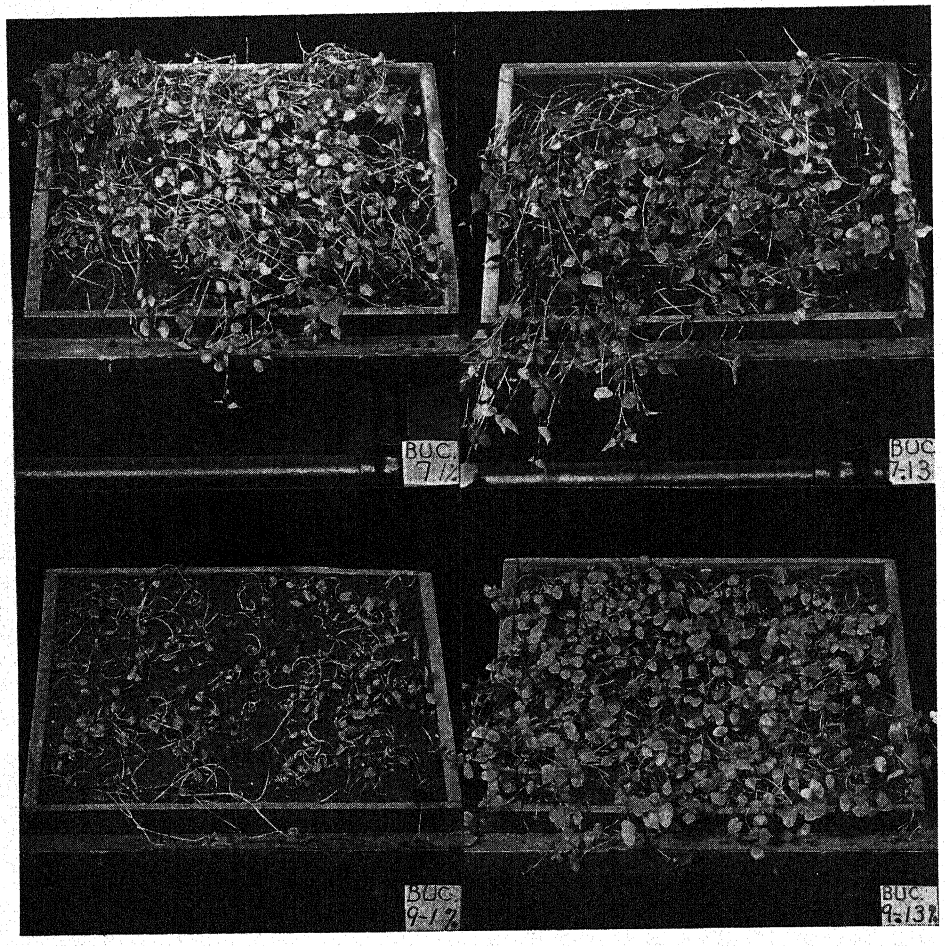


FIG. 11.—Buckwheat plants sprayed with lanolin emulsions (*top*) containing 1% (*left*) and 0.13% (*right*) of 4-chlorophenoxyacetic acid and with similar concentrations of 2,4,5-trichlorophenoxyacetic acid (*bottom*).

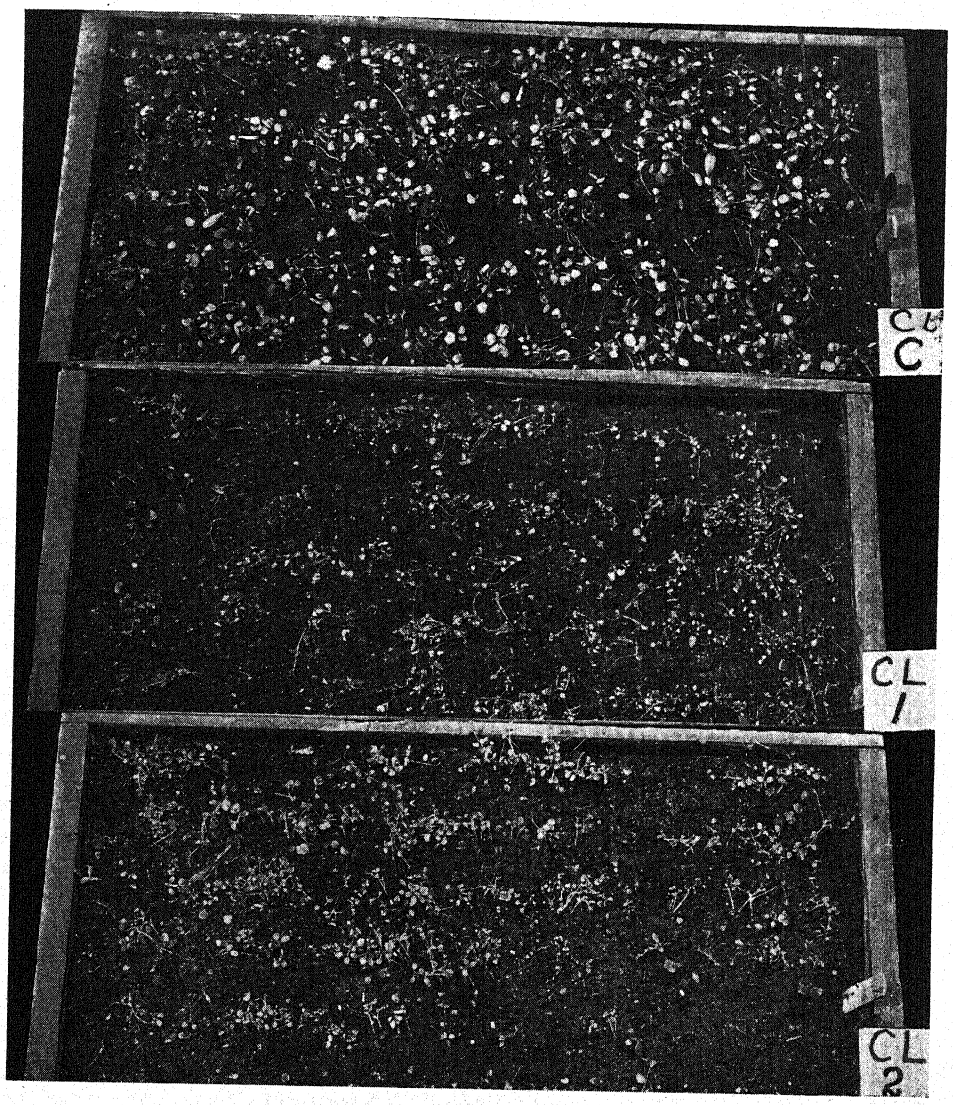


FIG. 12.—Clover plants 26 days old when treated. *Top*, untreated. *Center*, sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, sprayed with aerosol containing 4-chlorophenoxyacetic acid.

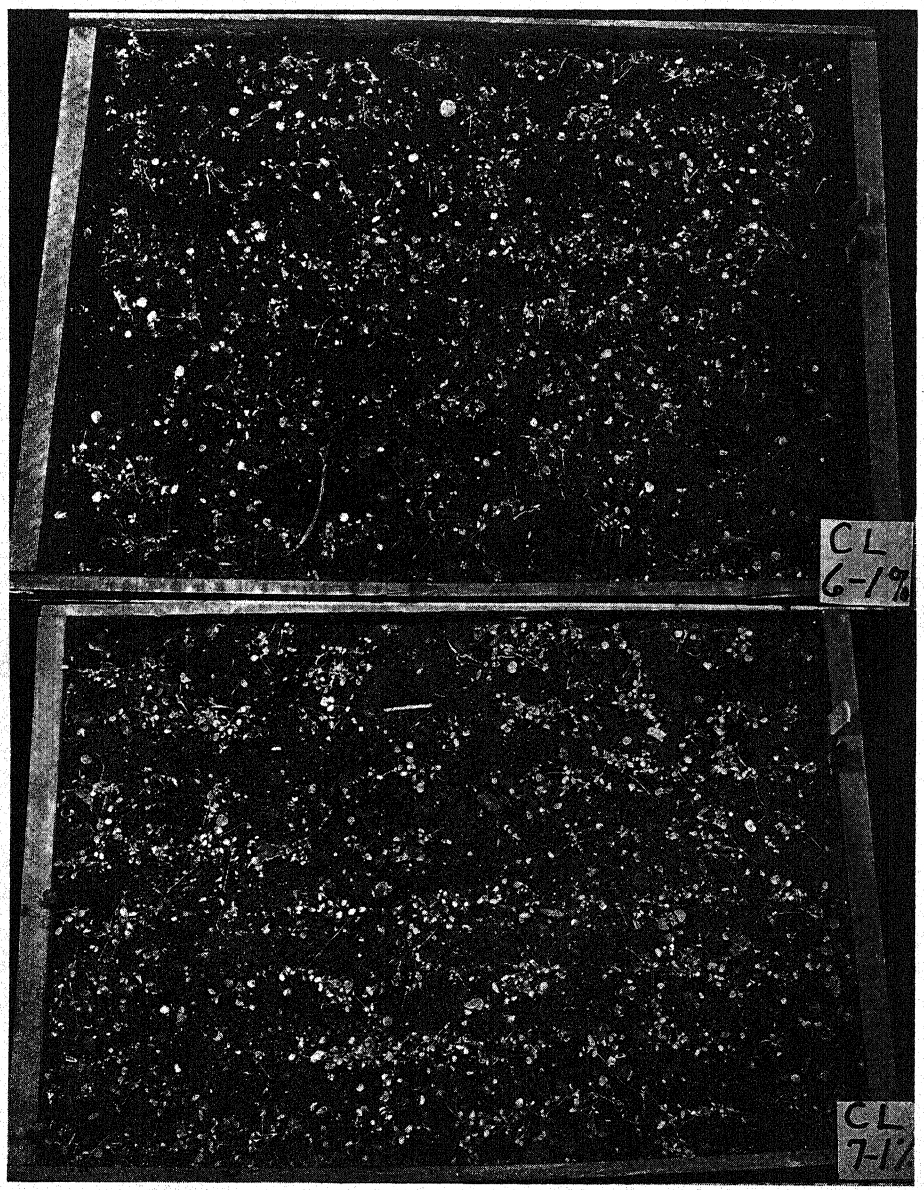


FIG. 13.—Clover plants sprayed with lanolin emulsions containing 1% of 2,4-dichlorophenoxyacetic (*top*) and 1% of 4-chlorophenoxyacetic acid (*bottom*).

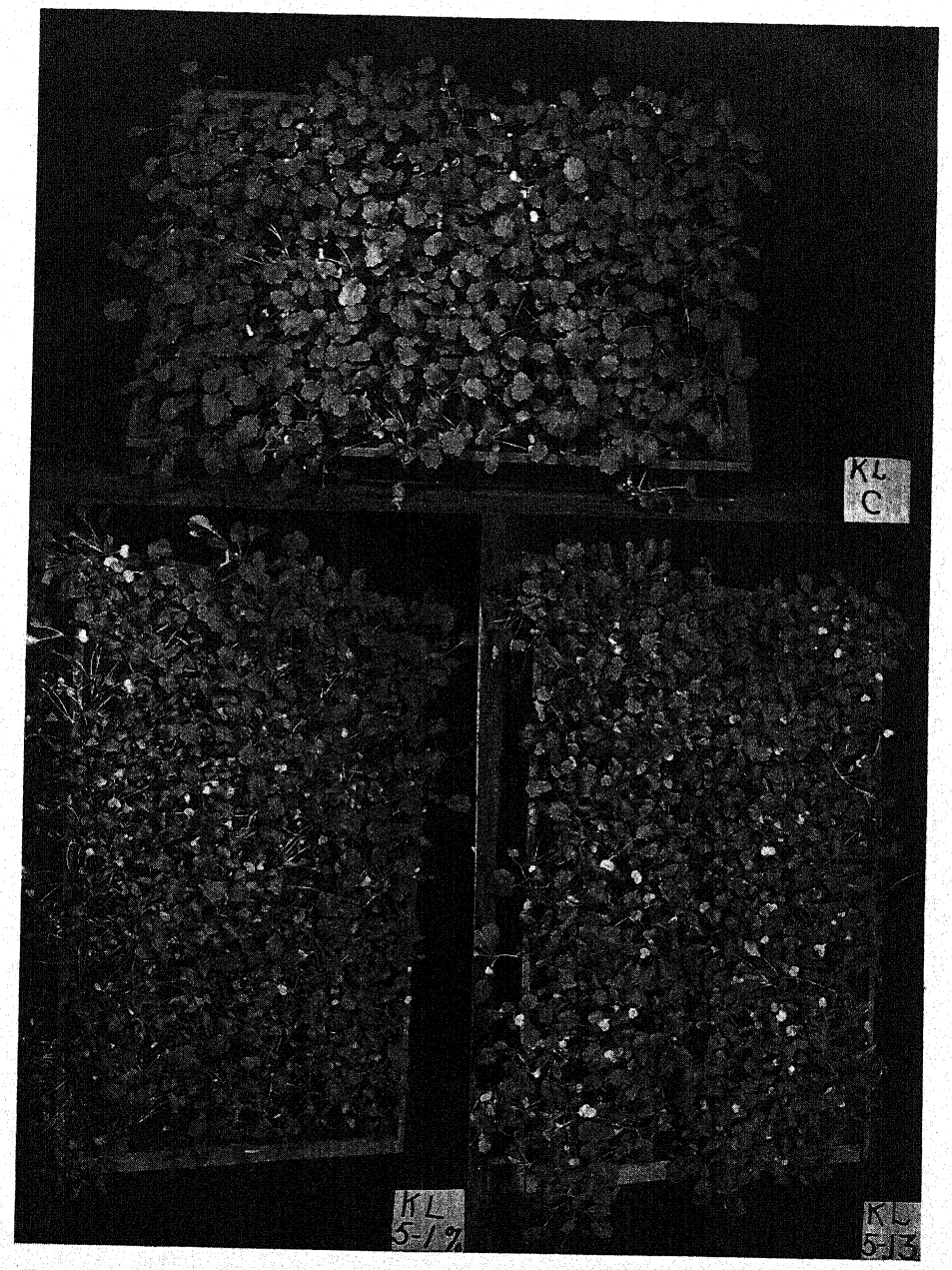


FIG. 14.—Rape plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with lanolin emulsions containing 1% (*left*) and 0.13% (*right*) of triiodobenzoic acid.



FIG. 15.—Rape plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid; 1% (*bottom, left*) and 0.13% (*right*) of 4-chlorophenoxyacetic acid.



FIG. 16.—Rape plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*right*) of 2-chlorophenoxyacetic acid; 1% (*bottom, left*) and 0.13% (*right*) of 2,4,5-trichlorophenoxyacetic acid.



FIG. 17.—Radish plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with an aerosol containing 2,4-dichlorophenoxyacetic acid (*left*) and 4-chlorophenoxyacetic acid (*right*).



FIG. 18.—Bean plants 26 days old when treated. *Top*, untreated. *Bottom*, sprayed with an aerosol containing 2,4-dichlorophenoxyacetic acid. Photographed 10 days after treatment. Upper plants served as "young" controls for other experiments.

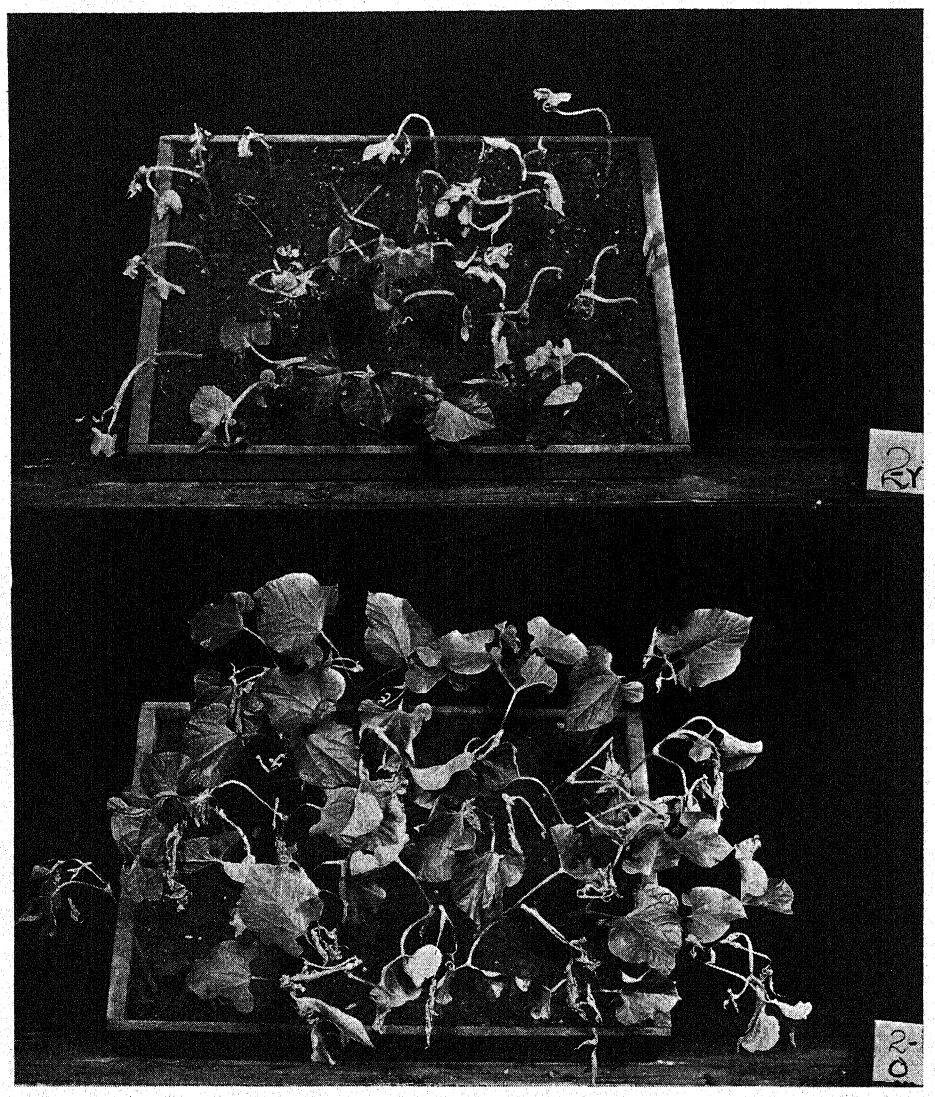


FIG. 19.—Bean plants. *Top*, sprayed 26 days after planting with an aerosol containing 6.3% of 4-chlorophenoxyacetic acid. *Bottom*, sprayed in similar way 44 days after planting. Note difference in response by plants of different ages.



FIG. 20.—Bean plants. *Top*, sprayed 26 days after planting with an aerosol containing 1.26% of pentachlorophenoxyacetic acid. *Bottom*, treated in similar way 44 days after planting. Photographs taken 10 days after treatment. Compare with fig. 19.

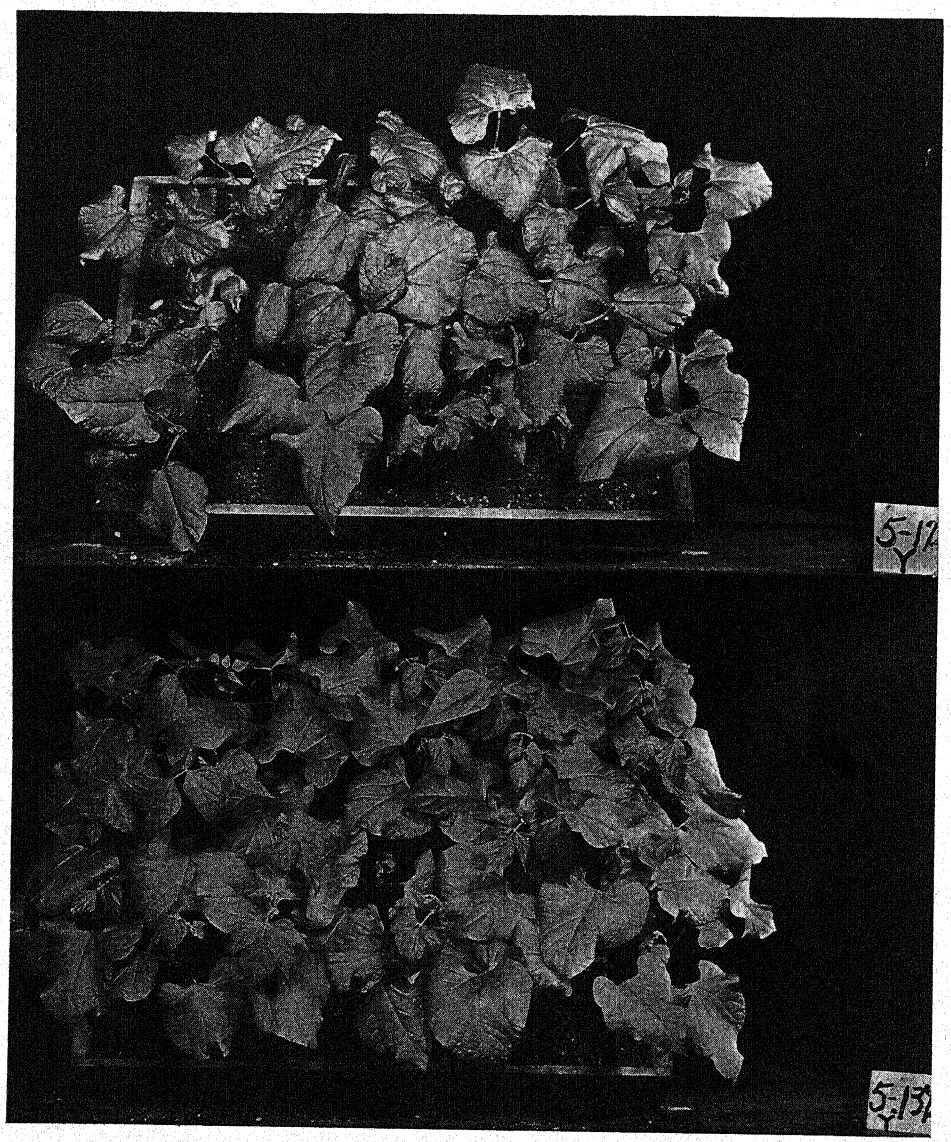


FIG. 21.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of triiodobenzoic acid.

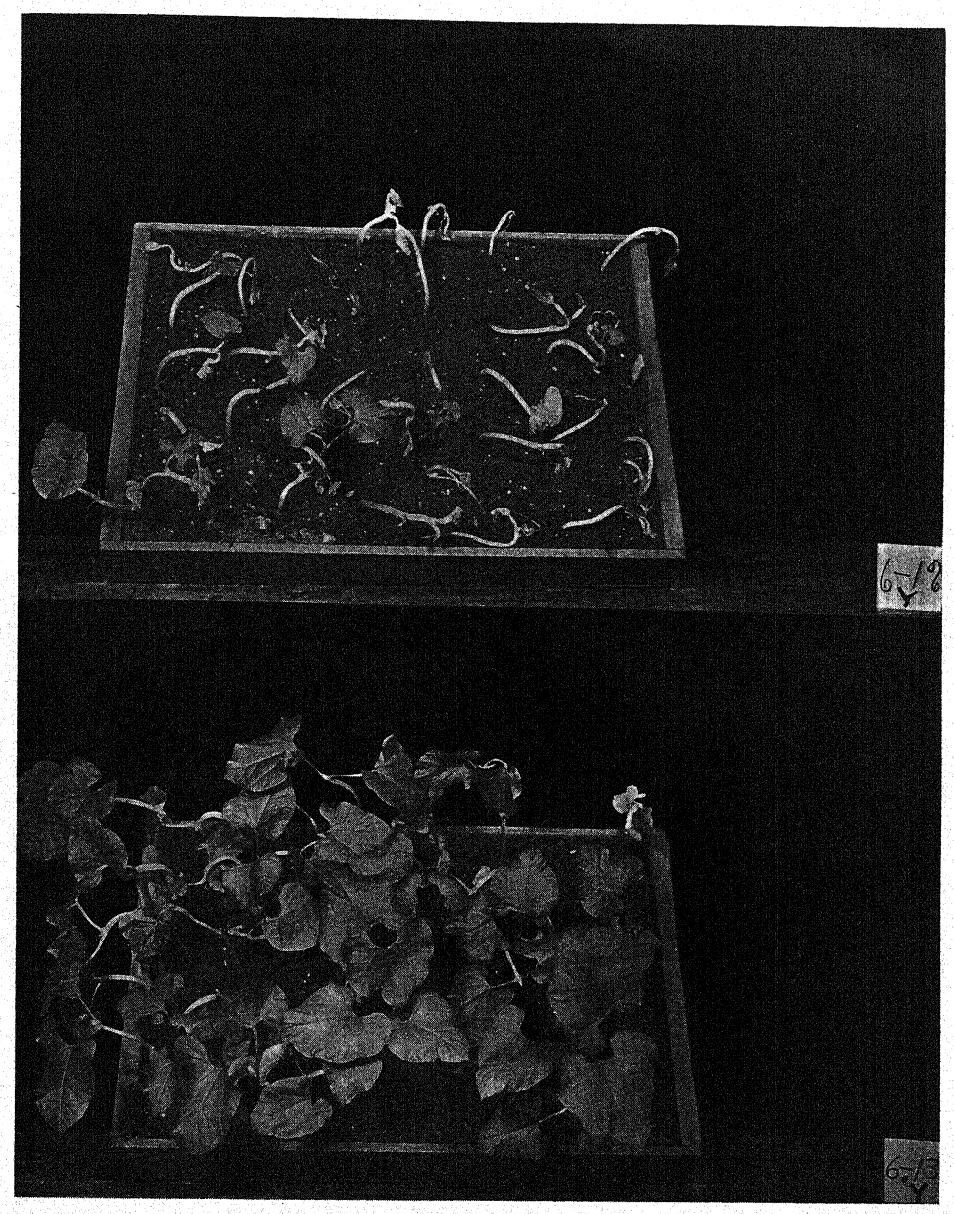


FIG. 22.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid.

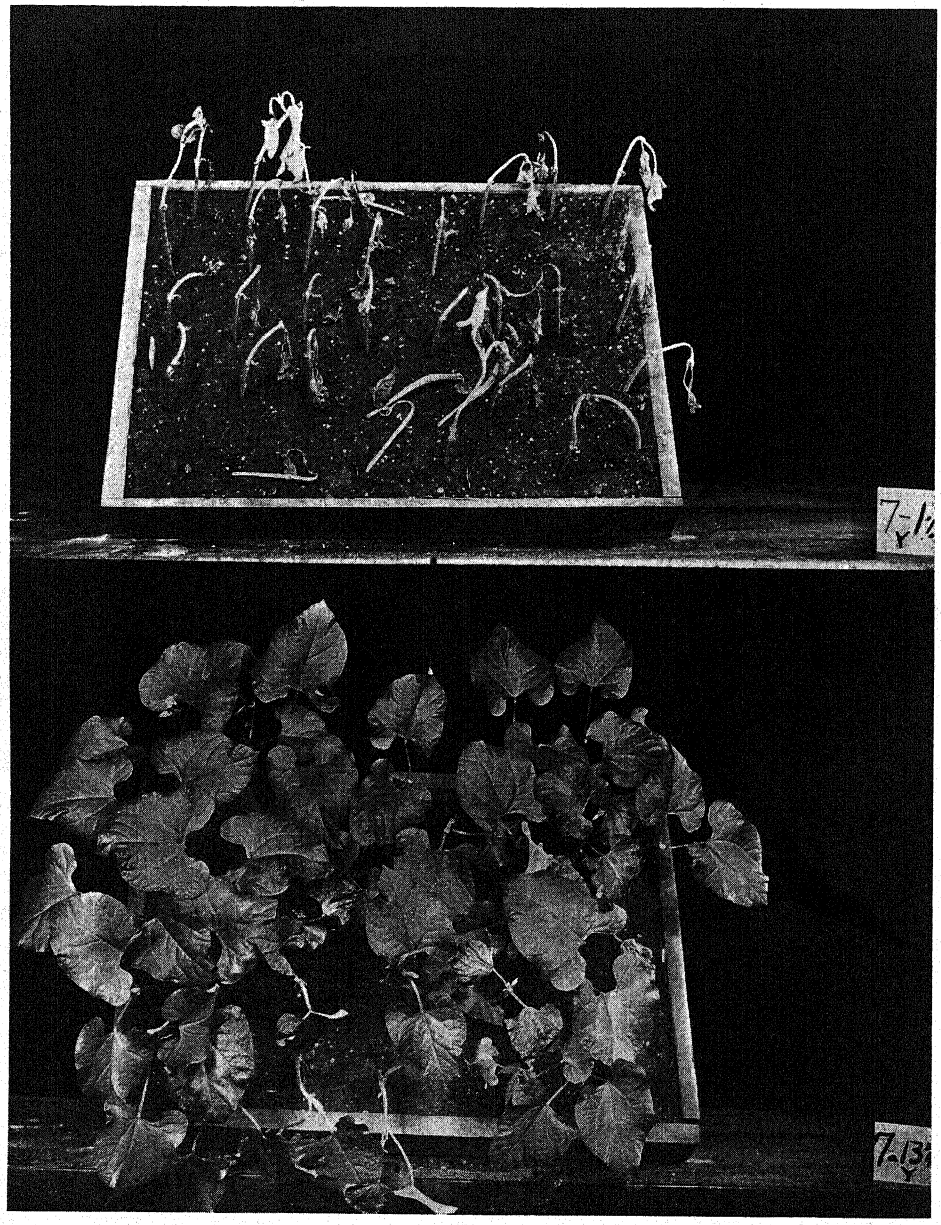


FIG. 23.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 4-chlorophenoxyacetic acid.

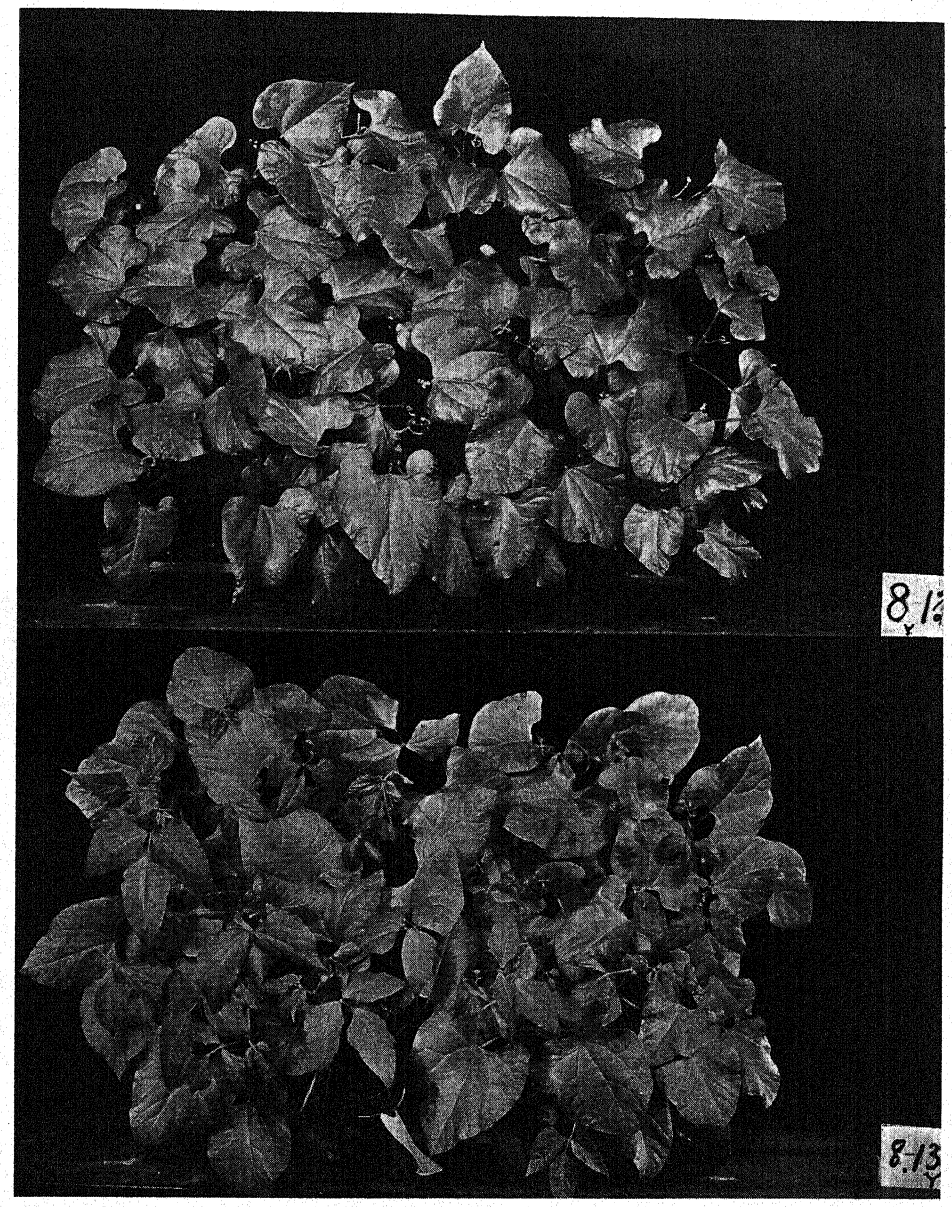


FIG. 24.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2-chlorophenoxyacetic acid.

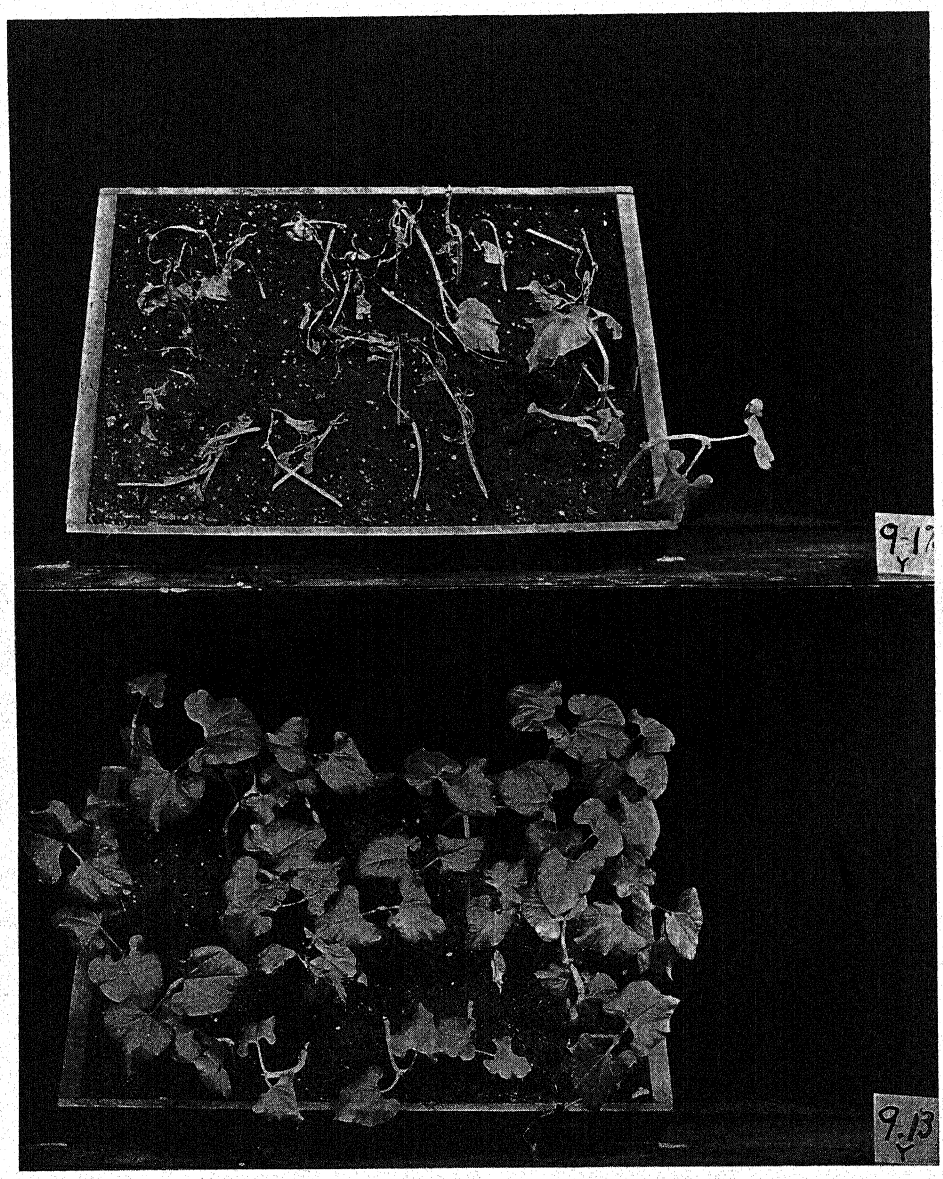


FIG. 25.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4,5-trichlorophenoxyacetic acid.

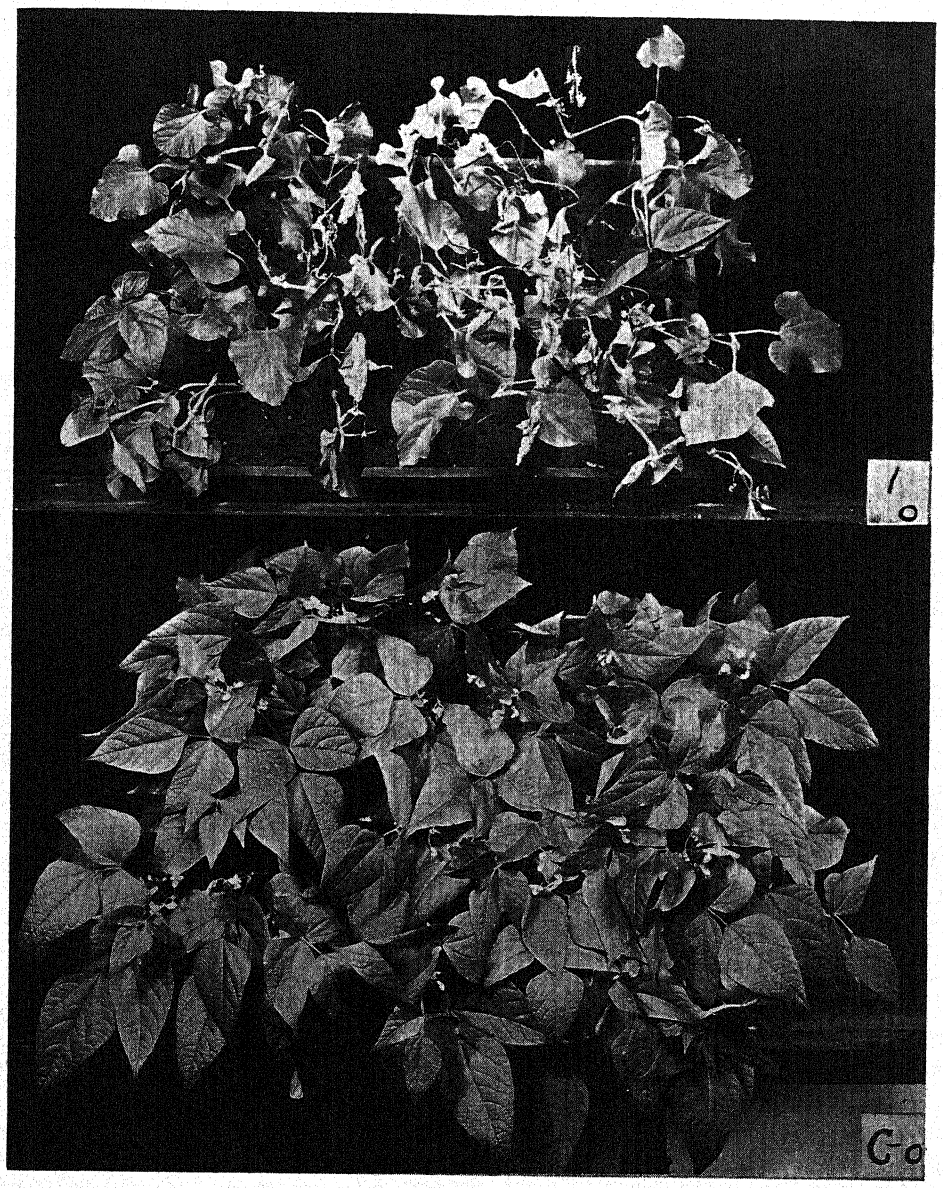


FIG. 26.—Bean plants 44 days old when treated. *Top*, sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, unsprayed. Photographed 10 days after treatment. Lower plants are referred to as "old" controls.



FIG. 27.—Bean plants sprayed 44 days after planting with aerosol containing 4-chlorophenoxyacetic acid (*top*) and penta-chlorophenoxyacetic acid (*bottom*).

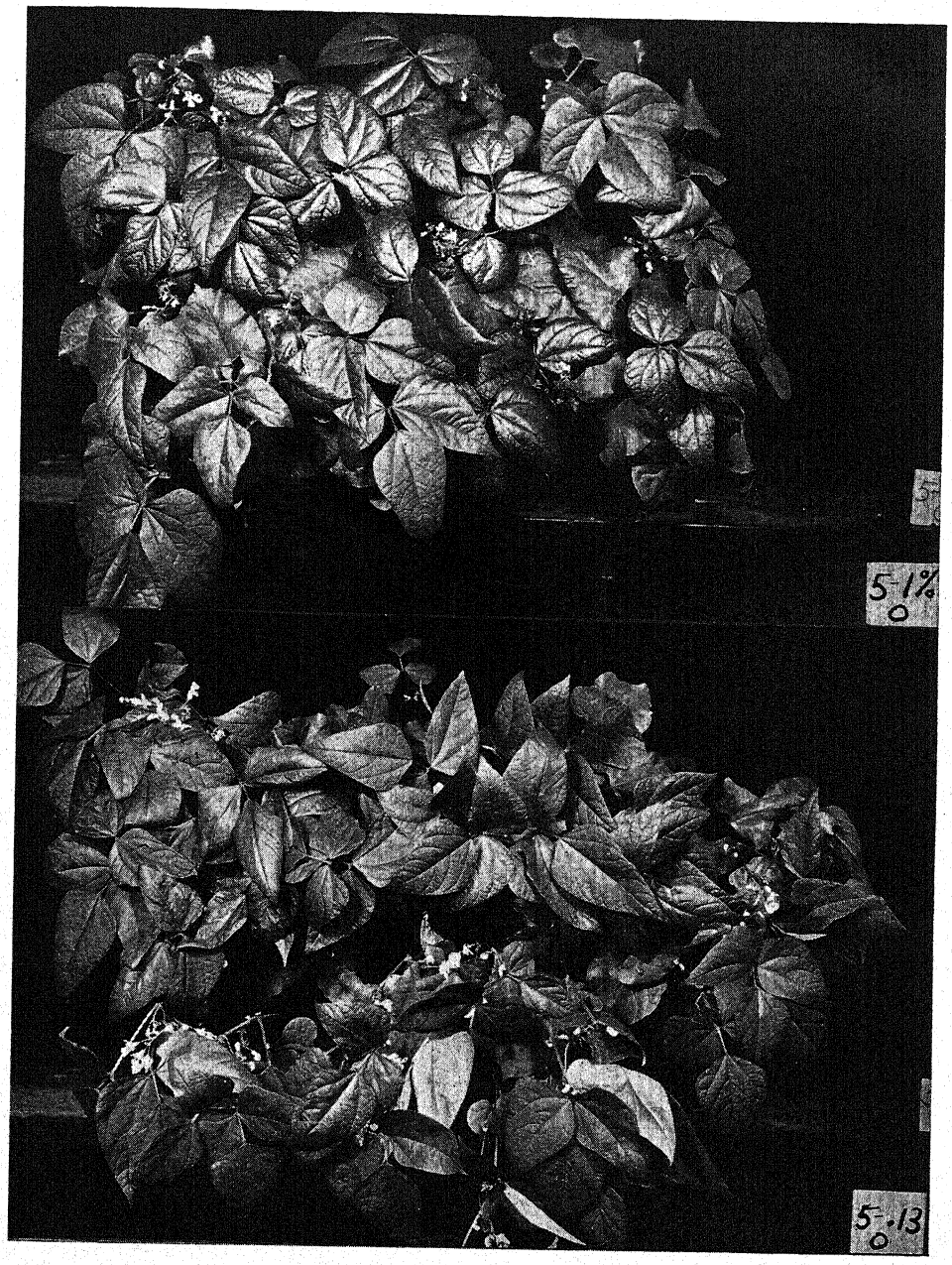


FIG. 28.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,3,5-triiodobenzoic acid.



FIG. 29.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 22.



FIG. 30.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4,5-trichlorophenoxyacetic acid. Compare with fig. 25.

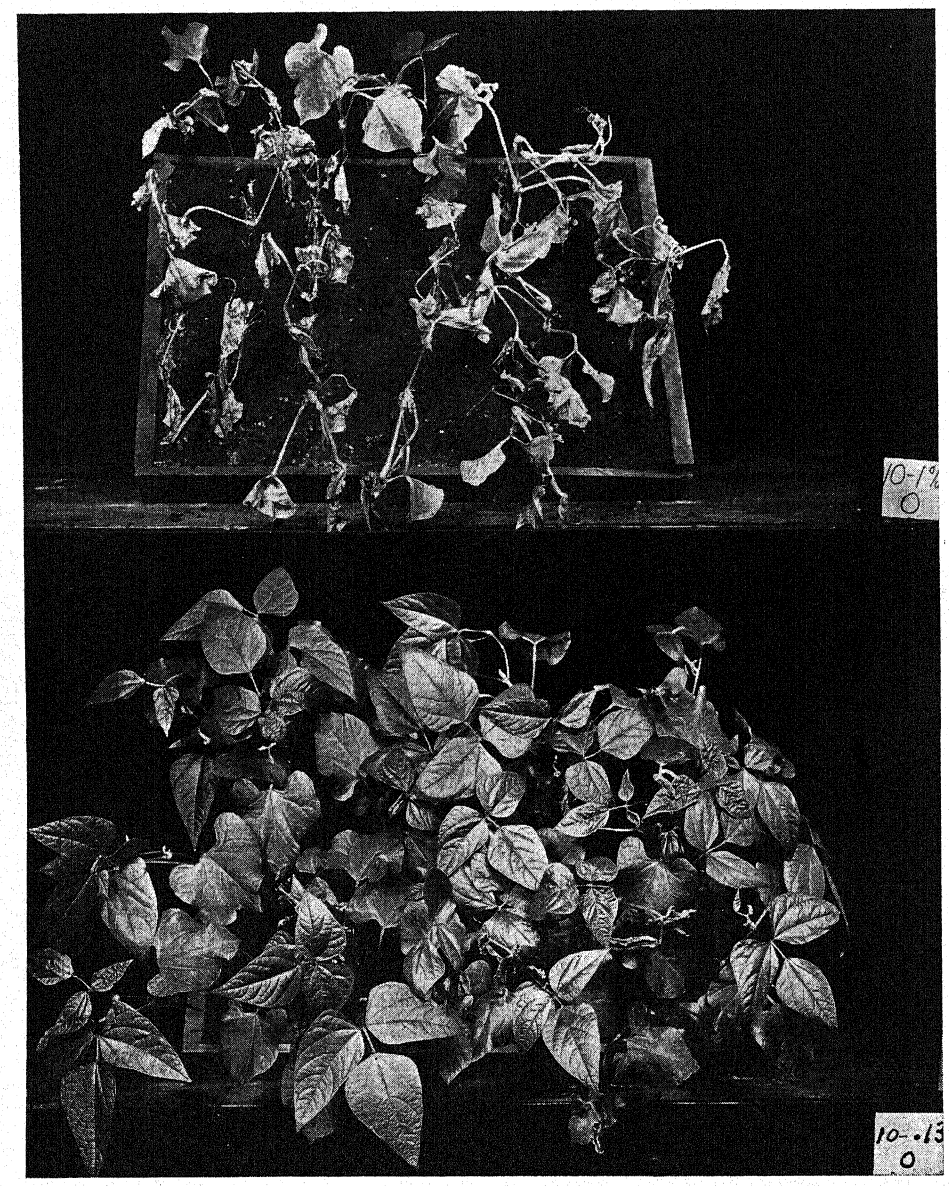


FIG. 31.—Bean plants sprayed 44 days after planting with heavy-oil emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 29.

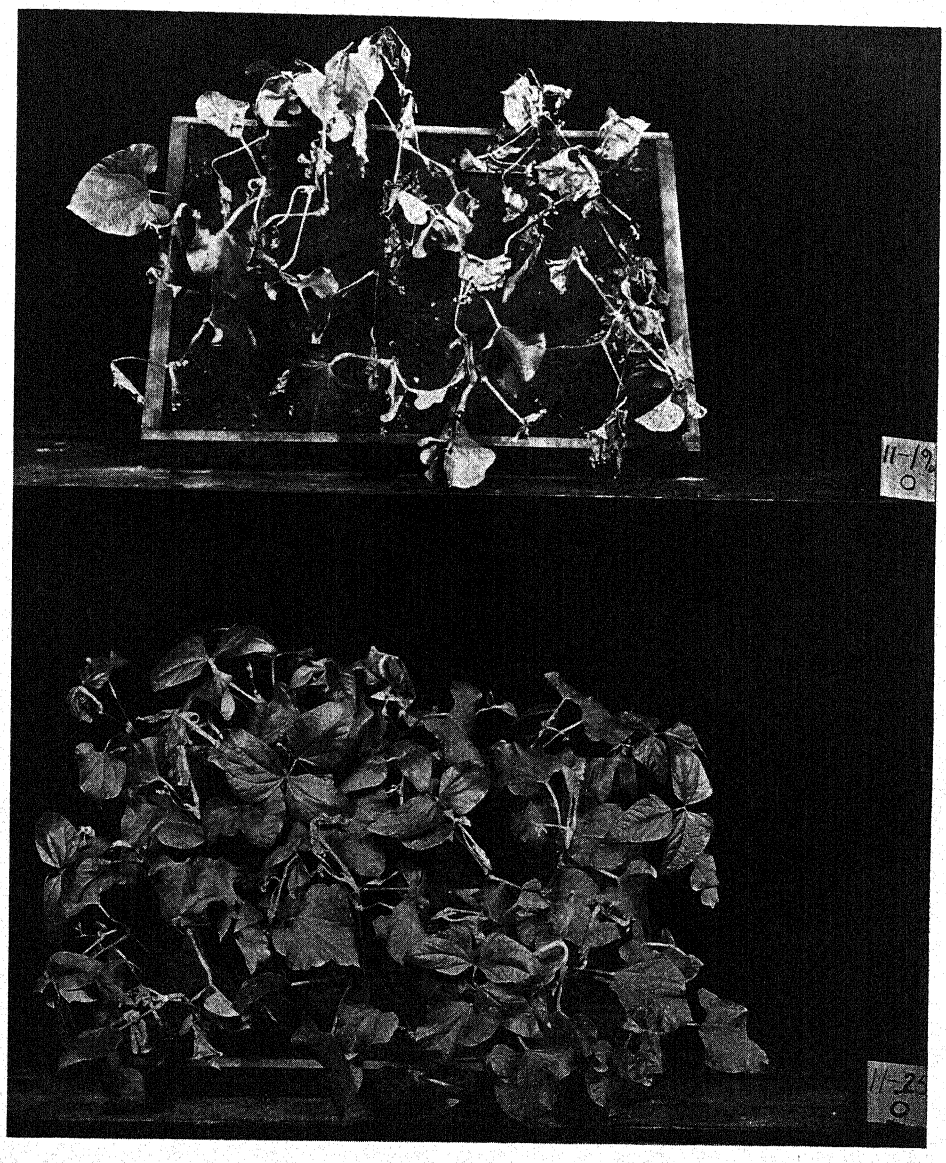


FIG. 32.—Bean plants sprayed 44 days after planting with light-oil emulsions containing 1% (*top*) and 0.25% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 31.

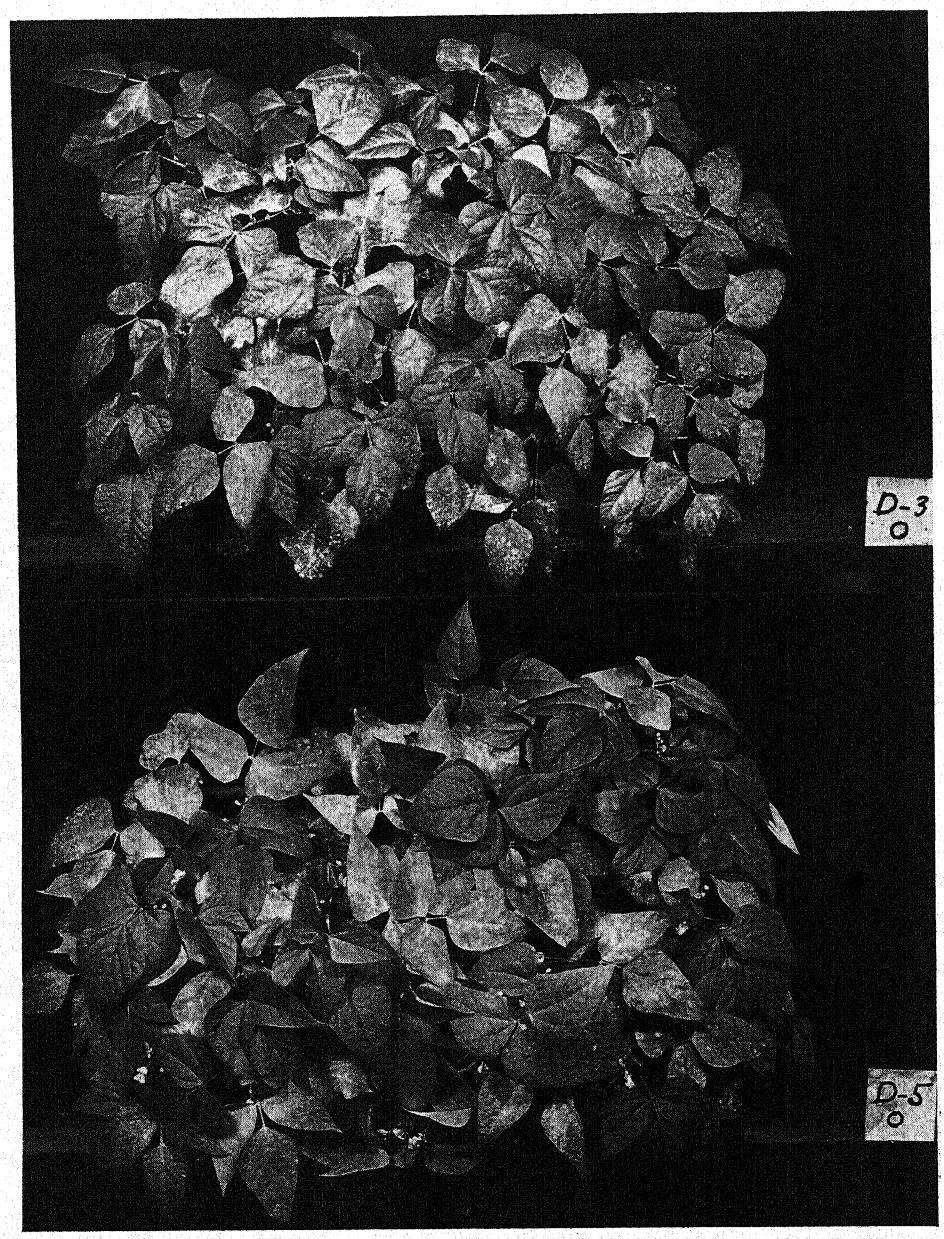


FIG. 33.—Bean plants dusted 44 days after planting with talc containing α-naphthyl methyl acetate (*top*) and penta-chlorophenoxyacetic acid (*bottom*). One part of growth-regulator to 500 parts talc.



FIG. 34.—Bean plants 44 days old when treated. *Top*, sprayed with lanolin emulsion containing 0.13% of 4-chlorophenoxyacetic acid. *Bottom*, dusted with talc containing 1 part of 2,4-dichlorophenoxyacetic acid to 500 parts of talc. Photographed 10 days after treatment. Some of effects which developed much later following treatment with dusts are discussed in text.

2,4-Dichlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, and penta-chlorophenoxyacetic acid were dispersed individually as aerosols in which the growth-regulating substances were carried in motor oil (S.A.E. 40). In producing an aerosol, 6.3 gm. of 2,4-dichlorophenoxyacetic acid were added to 50 ml. (approximately 50 gm.) of oil, and this mixture was dissolved in 150 gm. of dimethyl ether. The 4-chlorophenoxyacetic acid mixture was prepared by adding 3.15 gm. of the acid to 50 gm. of oil and dissolving this mixture in 150 gm. of dimethyl ether. To produce the third aerosol, 630 mg. of penta-chlorophenoxyacetic acid were added to 50 ml. of oil, and this mixture was dissolved in 150 gm. of ether. These mixtures were contained in small individual metal cylinders. In applying the aerosols, the cylinders were held approximately 2 feet above the uppermost leaves and directed downward so that the mist covered the entire flat of plants. Each flat was sprayed for a period of from 4 to 8 seconds.

Dusts were prepared that contained separately, α-naphthaleneacetic acid, α-naphthyl methyl acetate, penta-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid. Two concentrations of these agents were used, 1 part to 500 dust and 1 part to 5000 dust. In preparing a dust, the growth-regulating substance was first dissolved in alcohol. This solution was thoroughly mixed with the required amount of talc, and finally the paste was dried and repulverized, leaving the agent evenly distributed throughout the talc. The dust was applied to the above-ground portions of the plants by placing it in a cheesecloth bag and shaking the dust through the cloth onto the plants.

All treatments were applied on February 29, 1944. On the tenth day following treatment the photographic records were made. The plants which survived treatment were grown under greenhouse conditions for a period of from 1 to $2\frac{1}{2}$ months, during which time records as to their growth and fruitfulness were made. Some of these records are presented individually near the end of this paper; others are in the general summary of results given here.

Results of trial F

Toxicity.—2,4-Dichlorophenoxyacetic acid killed beet, rape, buckwheat, radish, clover, and young and relatively mature beans within 10 days when applied at a concentration of 1% in lanolin emulsion spray. Lower concentrations (0.5, 0.25, and 0.13%) of the acid in lanolin emulsions greatly retarded the growth of these plants but did not kill all of them. Both heavy- and light-oil emulsions containing 1.0 and 0.5% of this acid killed bean and beet seedlings, while the 0.25 and 0.13% concentrations greatly retarded growth but did not kill all the plants. Oil containing the acid and dispersed as an aerosol killed seedling buckwheat, bean, rape, beet, clover, and relatively mature beans and greatly inhibited the growth of radish seedlings. 2,4-Dichlorophenoxyacetic acid was equally toxic when applied either to the more mature or to the younger beet plants. Applied in concentrations of 1% in a light-oil emulsion spray, the acid killed rice of the varieties Blue Rose and Fortuna.

2,4,5-Trichlorophenoxyacetic acid killed seedling beet, clover, and the young bean plants within 10–15 days after treatment and greatly inhibited the growth of radish, rape, buckwheat, barley, and relatively mature bean plants when applied at a concentration of 1.0 and 0.5% in lanolin emulsion. An emulsion containing 0.13% of the acid in-

hibited the growth of rape, buckwheat, and bean plants.

4-Chlorophenoxyacetic acid killed clover and seedling bean plants within 10–15 days after treatment when applied at a concentration of 1% in lanolin emulsion and clover plants when applied at a concentration of 0.13% in lanolin emulsion. In a concentration of 1% in lanolin emulsion it greatly retarded the growth of seedling rape, beet, radish, buckwheat, and the more mature bean plants and caused appreciable injury to barley. The acid effectively retarded the growth of young bean plants when applied at a concentration of 0.13% in lanolin emulsion. Dispersed as an aerosol, it killed most clover plants so treated.

2,3,5-Triiodobenzoic acid noticeably retarded the growth of rape and young and more mature bean plants when applied at a concentration of 1% in lanolin emulsion. A 0.13% concentration of the acid, applied in lanolin emulsion, resulted in slight inhibition of the expansion of the trifoliate leaves of beans. Although this compound brought about very marked growth responses, particularly of the leaves, it failed to kill the different kinds of plants to which it was applied. After a period of time many of the plants treated with this compound not only recovered and bore fruit but remained vigorous for a considerable period after untreated controls of the same age had matured and died. Some of these results are recorded below.

Penta-chlorophenoxyacetic acid was applied in oil as an aerosol. It killed the majority of seedling clover plants so treated and injured some of the leaves of young corn plants, but it failed to retard their growth an appreciable amount. Applied in talc, it did not affect the growth of bean, beet, or barley plants.

2-Chlorophenoxyacetic acid brought about some plant-growth responses (epinasty and stem bending) but failed to inhibit to an appreciable amount the growth of most plants tested.

Talc containing either 2,4-dichlorophenoxyacetic acid, penta-chlorophenoxyacetic acid, α-naphthyl methyl acetate, or α-naphthaleneacetic acid was not effective in producing toxicity symptoms in beet, bean, or barley.

The growth of corn and wheat was not noticeably affected by the application of the various substances used in these experiments. The growth of oats was retarded slightly, and of barley appreciably so, by the application of 2,4,5-trichlorophenoxyacetic acid at a concentration of 1% in lanolin emulsion. Lower concentrations of the acid applied in the same way had little apparent effect. In general, cereals proved to be relatively insensitive to the various growth-regulating substances in the manner used. Rice, however, was killed by the application of 2,4-dichlorophenoxyacetic acid emulsion to the plants and to the water over the soil in which they were growing.

GROWTH RESPONSES.—Aside from toxic effects, various types of growth responses resulted from the application of certain of the substances used. Some of these responses are of significance here, since they altered the growth of the plants in such a way as to reduce greatly the amount of fruit produced or to extend the amount of time required for the plant to come to the fruiting stage.

Application of 2,4-dichlorophenoxyacetic acid, at a concentration of from 0.13 to 1.0% in lanolin emulsion, resulted in epinastic responses and stem curvatures within a period of 2 or 3 days following treatment. Growth of the terminal buds of the various dicotyledonous plants tested was greatly inhibited, especially when the acid was applied in

concentrations of 0.25% or more. Tumors developed in the stem tissues, and, as a result, the over-all growth of the plants was greatly reduced, as was the number of fruits which developed. With the exception of slight stem curvatures, no apparent growth response or form changes resulted when 2,4-dichlorophenoxyacetic acid was applied to the foliage of the cereals except as already noted for rice and possibly barley.

2,4,5-Trichlorophenoxyacetic acid also brought about very marked form changes when applied to the dicotyledonous plants as lanolin-emulsion or oil-emulsion sprays. These were similar in outward appearance to those described in connection with the use of 2,4-dichlorophenoxyacetic acid. The application of 2,4,5-trichlorophenoxyacetic acid to the cereals failed to bring about any significant changes of form except as noted for oats and barley.

When applied to the dicotyledonous plants, 4-chlorophenoxyacetic acid inhibited the growth of terminal buds to a very marked extent. The growth responses observed were similar in appearance and effect to those reported in connection with the use of 2,4-dichlorophenoxyacetic acid. The application of low concentrations (0.13–0.25%) of the acid to seedling clover plants resulted eventually in the development of leaves which had from three to nine leaflets and in the formation of numerous roots which emerged from the hypocotyls. Later the clover plants recovered completely and grew normally.

The application of 2,3,5-triiodobenzoic acid to the dicotyledonous plants resulted in the inhibition of internodal elongation and in modification of growth of the leaves in the terminal buds. The growth of lateral buds was affected in a similar way when emulsions containing 1% of the acid were applied. Leaves of the affected buds failed to expand the usual amount. They were very small and curled, forming rosettes while the plant was in the seedling stage. They expanded slightly as the plants matured. The leaves produced later were normal. In the case of the beet, many of the leaves produced after treatment were tubular, funnel-shaped, or filiform, with the surface much pebbled. 2,3,5-Triiodobenzoic acid failed to induce the formation of stem tumors on any of the plants. The number of flowers that developed on treated plants was not appreciably fewer than the number that developed on untreated ones. However, at the end of the experiment plants treated with a concentration of 1% of the acid in lanolin emulsion were somewhat smaller in over-all size than were untreated ones. The behavior of some of the plants when appreciably older is recorded below, under treatment 5. The application of the acid did not noticeably alter the growth of the monocotyledonous plants studied.

Many of the flats of plants in this trial which were not killed by the treatments were held over and placed on greenhouse benches for further development. It is obvious that several points of great agricultural importance are shown, especially the increased longevity of some of the lots of plants and the intense green coloration developed by the foliage following some of the treatments. These observations, especially as they relate to an apparent increase in development over the controls and give indications of a decided stimulative effect, are of as great significance as are the toxicity effects.

Except as specifically indicated the following notes on bean plants were made on April 17, 1944. The exceptions relate to a few flats on which the notes were

made on May 3, 1944. The numbers used designate the same type of treatments as given in table 1.

5. *1% (old).*—Yellowed; one set of trifoliate leaves normal, the others curled and crumpled, or pebbled. Few flowers, few pods, one to three beans each.

5. *0.5% (old).*—Yellowed; many large trifoliate leaves, many small axillary shoots; those at cotyledonary node show no crumpling and are dark-green. There are a few dry pods having one to three beans each, many large green pods having one to three beans each, and a number of flowers on the axillary shoots. In general, slightly greener than controls. The plants are not so green as treatment **8.** *Dust* nor so large.

5. *0.13% (old).*—Yellow, many plants mature and dried, a few with axillary shoots at the cotyledonary node. Most pods dry, one to two seeds each. Some large trifoliate leaves. Slightly less mature than controls.

5. *1% (young).*—Primary leaves large, yellowed, somewhat recurved; epicotyls with few green leaves or leaves dead and shed. Axillary shoots at cotyledonary node. Small, very green leaves, a few young green pods, one to three seeds each. Still many flowers and young pods forming.

5. *0.5% (young).*—Epicotyls dead, few axillary shoots, also a few shoots from axils of cotyledons. These shoots are still green. Leaves mainly yellowed or fallen, stems dead or dying. Plants sparse in appearance.

5. *0.25% (young).*—Yellowed, dying, epicotyls dead; a few had produced thin shoots with small leaves. A few pods with one bean in each. Weak sprouts from axils of cotyledons.

5. *0.13% (young).*—Yellowed; some second internodes are elongated and have few large leaves. Axillary shoots at nodes of cotyledons and primary leaves. Leaves small, pebbled, all yellowed, except those on small axillary shoots. Few green or dry pods. Less mature than controls.

6. *0.13% (young).*—Still green, the primary leaves green and somewhat leathery. Epicotyls killed, with large tumors, 1 inch in length, stopping at cotyledonary nodes where large tumors have formed on some plants. Small, green, axillary shoots at cotyledonary nodes, a few in axils of primary leaves. There are flowers only at these places. A few green pods, containing one to three beans each. Plants still actively vigorous; new green leaves show little deformation.

6. *0.13% (old) (May 3, 1944).*—Many axillary shoots, some of them with tumors, mostly without. Primary leaves not very large but still green. Short axillary shoots green, with green pods having one to three beans each. Larger trifoliate leaves mottled yellow. Plants rather open, not very bushy and still appear decidedly green. Less bushy than **7.** *0.13% (old).* More pods than controls, some of them still green, some dried.

7. *0.13% (young).*—No large trifoliate leaves. Those which are present still very green. Most of the terminal shoots were killed; others have large galls which are corky, many extending to the cotyledonary nodes, none below. The lateral shoots are dwarfed with some bloom, but most of the pods are aborted. Many leaves filiform; no compound leaves of full size. Most of the primary leaves still dark-green, leathery, but not of unusual size. Plants as a whole appear very green.

7. *0.25% (old).*—Still very green, except a few primary leaves and the old, full-sized, trifoliate leaves which are yellowed. Apical buds of main stems and also buds present in the axils of trifoliate leaves at time of spraying became tumors. Many new shoots in axils of primary leaves; most of them with deformed, sometimes filiform, leaves. These shoots are blooming and sometimes have green pods, many of which are abortive. Axillary shoots at cotyledonary node with very green leaves which are slightly deformed. Many plants in bloom and with large green pods having one to three beans each, although some are large and empty. Plants have a decidedly bushy appearance. They are vigorous except for many deformed leaves. General appearance very green with many buds and blossoms.

7. *0.13% (old) (May 3, 1944).*—Plants green and taller than **7.** *0.13% (young);* many large trifoliate leaves which are slightly yellowed. Tops and axillary shoots which were present at nodes of large trifoliate leaves when sprayed are now oval, fleshy, corky tumors with apical rosettes of scales. A few plants have much-branched shoots instead of tumors. These shoots have intense green leaves which are rather small and sometimes filiform. A few axillary shoots at cotyledonary nodes are mostly small with leaves less deformed. Many large green pods, one to five beans each. Some flowers and a few buds.

8. *1% (young).*—Greener than plants treated at 0.5% strength. Axillary shoots with many

small deformed leaves. A few abortive flowers and pods. Some buds with one to two seeds each. Many leaves filiform. General appearance very twiggy.

8. *0.5%* (*young*).—Yellow. Epicotyls thin, dying, with many of the leaves filiform or with broad midveins and no blade. Weak shoots in axils of cotyledons.

8. *0.25%* (*young*).—Yellow, not so mature as controls. Epicotyls slender; many leaflets of trifoliate leaves narrowly triangular, deformed, and pebbled. Pods dry, one to two beans each. Stems green.

8. *0.13%* (*young*).—Yellow, but less so than controls. Epicotyls slender; leaves small, slightly deformed. Very little branching from axils of cotyledons or leaves. Few pods, usually with one seed.

8. *1%* (*old*).—Greener than controls, but plants are not so green as treatment 8. *Dust*. Many large trifoliate leaves, some of which are yellowed, others very green. Many small green axillary shoots.

8. *0.5%* (*old*).—Not much deformation of plants, but they are much greener than controls. Stems green. A few dry pods.

8. *0.25%* (*old*).—Similar to *0.5%* (*old*) but still greener.

8. *0.13%* (*old*) (*May 3, 1944*).—Plants open, sparse, about like controls, but slightly greener. Many green pods, a few of them mature except at nodes of primary leaves and cotyledons.

11. *0.13%* (*old*).—Still much greener than controls. Second internode and internodes above elongated in many cases. A few have tumors on them. Leaves not much deformed, some trifoliate leaves large and green. Very few pods; most of them are green, with one to three seeds each. Stems green. Some tumors on petioles of older leaves.

1. *Dust*.—Slightly greener than controls; apparently more pods.

2. *Dust*.—Similar to treatment 1. *Dust* but more small, dark-green, axillary shoots.

3. *Dust*.—Greener than controls, more pods which are still fairly green, three to five beans each. Stems green. Compared with controls, less mature, slightly more vigorous.

7. *Dust*.—Plants greener than controls, many still growing actively. There are a few dry pods and some green ones having one to four seeds each. More growth than controls and still very much greener.

8. *Dust*.—Plants larger than controls, greener, and with many small axillary shoots. More pods, some of them still green. A few flowers still present.

Control (*young*).—Plants somewhat green, but primary leaves are yellow, as are also many of older trifoliate leaves. Stems still green. Few dry pods; few axillary shoots, those present being at nodes of cotyledons or primary leaves.

Control (*old*).—Plants yellow; most of primary leaves curled or shed, the large trifoliate leaves yellowish. Many mature pods, one to four seeds each. Stems collapsing and dying, mostly fallen over.

Summary

1. On the basis of the records of many experiments which demonstrated the relative toxicity of some of the growth-regulating substances and the differential sensitivity shown by plants in relation to them, the suggestion was made that greater attention be directed toward more critical study of these effects and their possible significance to agricultural and industrial practice. Following out this idea, it was specifically suggested that attention be given to the possible significance of these compounds in a program for national defense.

2. Experiments, in addition to work in progress, looking to both ends were carried out for a number of years. Summary results of certain of the specific experiments carried out from August, 1941, to May, 1944, are given. Others may be presented at some future time.

3. When attention was called to some of the chlorophenoxyacetic and iodobenzoic acids, additional critical experimental work was at once centered around some of them.

4. The 2,4-dichlorophenoxyacetic, 2,4,5-trichlorophenoxyacetic, and 4-chlorophenoxyacetic acids proved extremely toxic when applied under greenhouse conditions to the aerial parts of sugar-beet, radish, rape, kidney bean, red clover, and buckwheat plants. Emulsions of these compounds, when applied to the foliage of rice plants and to the water

over the surface of the soil in which they were growing, were highly toxic.

5. Various other substances were effective as plant toxicants or in modifying growth when sprayed on the plants as lanolin or oil emulsions or when used as aerosols.

6. The concentrations of these agents required to kill or markedly to reduce the rate of growth of the plants ranged between 0.13% and 1.0% when the acids were applied as emulsion sprays. Penta- and 2-chlorophenoxyacetic acids were less toxic than 2,4-dichlorophenoxyacetic, 2,4,5-trichlorophenoxyacetic, and 4-chlorophenoxyacetic acids.

7. Dusts containing growth-regulating substances were relatively ineffective as applied in these tests. It was suggested that, on the basis of these results, the addition of a hygroscopic agent to the mixture of talc plus a growth-regulating substance might allow for the absorption of moisture and thereby place a sufficient amount of the agent in a form to induce growth responses and even severe injury.

8. In general, cereals were relatively insensitive to growth-regulating compounds as used in these experiments. Rice was killed by the application of 2,4-dichlorophenoxyacetic acid to the water surrounding the plants as well as to their leaf surface. Barley also proved to be somewhat sensitive to some of the mixtures but less so than most of the dicotyledonous plants tested.

9. Aside from the toxic effects, it was established that the application of 2,4-dichlorophenoxyacetic acid, and some of the other substituted chlorophenoxyacetic acids, brought about responses by the dicotyledonous plants that were outwardly different in appearance from those that resulted from the application of 2,3,5-triiodobenzoic acid. On the basis of the results obtained, it appeared that 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids would be outstandingly useful as herbicides or as differential herbicides.

10. In connection with the experimental work then under way, in March, 1944, plots were laid out at Beltsville, Maryland, to study the use of growth-regulating substances as herbicides when applied to field-grown plants.

11. The results of the various experiments carried out, together with many photographs, some of which accompany this paper, were conveyed to those authorized to receive such reports (13).

12. The use of growth-regulating substances in the control of weeds and other vegetation has now become widely publicized and exploited. Some of the other results of these experiments also are finding their application in agricultural and industrial practice.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
AND
BUREAU OF PLANT INDUSTRY, SOILS
AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

LITERATURE CITED

1. ANONYMOUS. Synthetic plant hormones. Science Suppl. **95**:12. 1942.
2. ———. Plant growth regulators. Special Projects Division, Chemical Warfare Service. Science **103**:469–470. 1946.
3. ———. Science and life in the world. George Westinghouse Educational Foundation Forum, May 16–18. Science **103**:662–666. 1946.
4. GRACE, N. H. Physiologic curve of response to phytohormones by seeds, growing plants, cuttings and lower plant forms. Canadian Jour. Res. **15**:538–546. 1937.
5. HAMNER, C. L. Physiological and chemical responses of bean and tomato plants to alpha-naphthalene acetamide and phenylacetic acid. BOT. GAZ. **103**:374–385. 1942.

6. HAMNER, M. E. Effect of phenylacetic acid and naphthalene acetamide on tomato plants grown in soil. BOT. GAZ. 103:576–580. 1942.
7. KRAUS, E. J. Histological reactions of bean plants to l-tryptophane. BOT. GAZ. 102:602–622. 1941.
8. KRAUS, E. J., and MITCHELL, J. W. Histological and physiological responses of bean plants to alpha-naphthalene acetamide. BOT. GAZ. 101: 204–225. 1939.
9. MERCK, G. W. Peace-time implications of biological warfare. *In* Science and life in the world. 2:129–146. McGraw-Hill Book Co., New York, 1946.
10. MITCHELL, J. W. Effect of indoleacetic acid on the growth of some crop plants. Proc. Amer. Soc. Hort. Sci. 36:171–176. 1939.
11. MITCHELL, J. W., and RICE, R. R. Plant-growth regulators. U.S. Dept. Agric. Misc. Pub. No. 495. 1942.
12. MITCHELL, J. W., and STEWART, W. S. Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. BOT. GAZ. 101:410–427. 1939.
13. NORMAN, A. G. Studies on growth-regulating substances. BOT. GAZ. 107:475. 1946.
14. PEARSE, H. L. The effect of phenylacetic acid and of indolebutyric acid on the growth of tomato plants. Jour. Pom. and Hort. Sci. 14: 365–375. 1936–37.
15. SLADE, R. E.; TEMPLEMAN, W. G.; and SEXTON, W. A. Plant-growth substances as selective weed killers: differential effect of plant-growth substances on plant species. Nature 155:497–498. 1945.
16. STUART, N. W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. BOT. GAZ. 100:298–311. 1938.
17. TEMPLEMAN, W. G., and MARMOY, C. J. The effect upon the growth of plants of watering with solutions of plant-growth substances and of seed dressings containing these materials. Ann. Appl. Biol. 37:453–471. 1940.
18. TEMPLEMAN, W. G., and SEXTON, W. A. Effect of some arylcarbamic esters and related compounds upon cereals and other plant species. Nature 156:630. 1945.
19. ZIMMERMAN, P. W. The formative influences and comparative effectiveness of various plant hormone-like compounds. Torreya 43:98–115. 1943.
20. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–343. 1942.

THE COMPARATIVE MORPHOLOGY OF THE FAGACEAE
I. THE GENUS NOTHOFAGUS[1]

LADEMA MARY LANGDON

Introduction

Although the anatomy and floristic features of the Betulaceae have been studied more or less intensively by a number of investigators, including recent detailed treatment of their inflorescence anatomy and morphology by ABBE (1, 2), similar studies involving the beech-oak-chestnut branch of the Fagales have been, since the early taxonomic and phylogenetic treatises (16, 18, 29, 30, 34), conspicuously meager. RENDLE (33), SCHWARTZ (35), and CAMUS (12) gave more complete comparative accounts of generic differences in this family than are found in other recent studies of the group, but in all three—as in earlier accounts—there is notable an inadequate, almost superficial, treatment of other than external taxonomic characters and a genuine scarcity of reliable material illustrative of the internal morphology of either the vegetative or the reproductive structures.

BENSON's conclusions, based upon such features of the megagametophyte as could at the time (4) be ascertained in the Betulaceae and Fagaceae, confirmed

[1] A part of this report was presented before the General Section of the Botanical Society of America at Boston, December, 1946. The investigation has been aided by grants from the Penrose Fund of the American Philosophical Society.

EICHLER's (16) and PRANTL's (29, 30) deductions based on their floral morphology, i.e., that the Cupuliferae were to be regarded as less specialized than the Betulaceae. This view has received support from anatomical studies (3, 37) as well as from more recent investigations in the floral morphology of certain of the Castaneae (6). In general, the more recent researches in inflorescence anatomy and morphology in families of the Amentiferae (8, 19, 22, 27) have emphasized specialization in these forms, and reduction, either from the Ranalian stock along the line of the Celastrales and the Sapindales or from the Rosales through the Hamamelidales.

RENDLE's taxonomic studies (33), on the other hand, support the theory—as the one most in accord with present knowledge—that the Fagales, along with the Casuarinales and Juglandales, are surviving representatives of diverging lines of development from some early angiospermous type or types now extinct, very likely with bisexual hypogynous flowers and bractlike perianths. On the basis of his studies, ABBE (1, 2) has proposed, as a hypothetical type ancestral to the Betulaceae, a form characterized by a series of racemously arranged cymose inflorescences, each composed of a median and two lateral triflorous groups, the individual flowers hermaphroditic and epigynous. The Fagaceae are mentioned as exhibiting a combination of characters suggestive of this type.

The comparative survey of the sporophytic characters of species of the existing genera of the Fagaceae, of which the present studies of *Nothofagus* are a section, has, as its major objectives, a clearer definition of the generic characters of the entire beech-oak-chestnut assemblage as well as the acquisition of additional data bearing on the ancestry of the amentiferous angiosperms. For some time it has been recognized that there is no group of flowering plants in which greater confusion exists in relation to the limits of genera and species than the Fagaceae; and it has become increasingly evident that thoroughgoing morphological investigations, involving internal as well as external characters, would be of material assistance in indicating clear lines of distinction by which one may legitimately break up large complexes in this family into a reasonable number of distinct genera.

DISTRIBUTION OF NOTHOFAGUS

The genus *Nothofagus*, formerly considered to be a section of the genus *Fagus* —designated *Nothofagus* (false beech) by BLUME (9), BENTHAM and HOOKER (5), SOLEREDER (38), and KRASSER (23) and proposed as a genus distinct from the northern section of the beeches by the Swedish botanist OERSTEDT (28)—has been for many years recognized as comprising those beeches dominating the forests of ridges and valleys from 4000 to 700 meters altitude in extratropical South America, Australia, and New Zealand. Exclusive of the species recently reported from the Netherlands New Guinea, sixteen species have been recognized as valid in this genus; eight occur in antarctic South America, five in New Zealand, and three in southeastern Australia and Tasmania.[2] Under favorable

[2] Two theories have been current relative to the origin of the northern and southern branches of the Fageae, both receiving support from the data of paleobotany: (*a*) the hypothesis supported by BERRY (7) of a common ancestry for the northern and southern beeches and the geographical divergence of these two branches from a single area—very probably western Asia—culminating in the structural differentiation and geographical segregation characterizing the living representatives of *Nothofagus;* (*b*) the alternative hypothesis of an independent emergence of *Nothofagus* on the Antarctic continent and its northward migration by

conditions of moisture, temperature, and exposure to wind, *Nothofagus* becomes a large tree 90–200 feet in height, with a trunk 2–5 feet in diameter, frequently flanged or spur-buttressed at the base, the latter character being especially well developed on those evergreen species growing in forests subject to very heavy rainfall (14, 15, 31). As in other members of the beech-oak-chestnut family, however, species of *Nothofagus* growing at the higher altitudes gradually become dwarfed as they ascend until, above the 400-meter elevation, they form stunted forests of shrublike trees.

An interesting altitudinal zonation marks the distribution of the beeches of New Zealand (14). *Nothofagus solandri* (Hook. f.) Oerst. and *N. truncata* (Col.) Cockayne occur chiefly as lowland-montane forms, *N. fusca* (Hook. f.) Oerst. generally occupies an intermediate range up to 1000–2000 meters elevation, and *N. cliffortioides* (Hook. f.) Oerst. and *N. menziesii* (Hook. f.) Oerst. frequently ascend into the upper subalpine belt, even to the timber line.

In antarctic South America a latitudinal as well as an altitudinal zonation is associated with the distribution of the beeches, which have been reported (15, 17, 31) from a point on the west coast of Chile about latitude 33°, southward through western Patagonia and Tierra del Fuego. The most northern species is *N. obliqua* (Mirb.) Blume, which, according to ELWES and HENRY (17), was one of the principal trees in the primeval forests formerly clothing the lower slopes of the Andes at about latitude 35°. In the forest country south of the Maule River, *N. obliqua* occurs in association with *N. dombeyi* (Mirb.) Blume, these two species providing the principal timber supply of Chile. In the forests of Chillan (lat. 37°), *N. obliqua* grows up to 4000–5000 feet and is replaced at the higher elevations by *N. antarctica* (Forst.) Oerst. and *N. pumilio* (Poep. et Endl.) Krasser, the former being one of the widely distributed species, especially at high elevations, on both the Chilean and the Argentine sides of the Cordilleras from latitude 38° to Tierra del Fuego. *Nothofagus procera* (Poep. et Endl.) Oerst., a species illustrated and described in some detail in this paper, is one of the less common of the South American beeches. It is scattered in the forests of Chile (lat. 35°–36°), where it exhibits about the same altitudinal range as *N. obliqua*.

Recently, important additions to the beeches of the southern hemisphere—extending their present range of distribution from the antarctic to the highlands of the tropical zone[3]—have been reported from the collections of the Archbold New Guinea expeditions of 1937–1938, constituting part of the Netherlands New Guinea Herbarium of the Arnold Arboretum of Harvard University. These beeches, the first *Nothofagus* species to be reported from the East Indian area, are described by collectors BRASS and VERSTEEGH as large trees 30–40 meters in height, with trunks over 1 meter in diameter, dominating the forests of ridges and valleys from ±3100 meters altitude down to 2300 meters in the "Bele" River section northeast of Lake Habbema (lat. approximately 4.09° S.).

Certain of these interesting collections

three separate routes into South America, Tasmania, Australia, and New Zealand.

A recent proposal by the Russian geographer WULFF (41) is that a primary type, ancestral not only to the beech line but also to the chestnuts and possibly the oaks, may be represented in the modern flora by the genus *Pasania*, which includes more than a hundred species distributed in the islands of the Malay Archipelago.

[3] CAIN (11) has classified the Fageae (*Fagus* and *Nothofagus*) as among forms illustrating a type of bipolar distribution with species occupying essentially boreal and austral centers but completely absent from intervening tropical stations.

have been made available for examination and description in connection with this study,[4] and three different species are illustrated either in habit (figs. 14–17) or in details of anatomical structure (figs. 19, 28–33, 34–37).

Investigation

Material and methods

Although the distribution and general ecology of the known species of southern beeches, as also their taxonomic problems, have been dealt with by several investigators (5, 9, 10, 14, 15, 17, 20, 21, 28, 31, 32, 39, 41), very little, other than of their external taxonomic characters, is known about the morphology of these important members of the beech series—even less, in fact, than in other sections of the Fagaceae.

Most of the specimens furnishing the basic materials for the present studies were secured as loans from the extensive herbaria of the Arnold Arboretum and from the Gray Herbarium of Harvard University. Herbarium specimens have been supplemented by shipments from the Golden Gate Park, San Francisco, California, and by two sets of fixations from the Royal Botanic Gardens, Kew, England, and from the Royal Botanic Garden of Edinburgh, Scotland.

For the histological and anatomical features of inflorescences, flowers, and fruits substantially the same technical procedure has been followed in imbedding, serial sectioning, and staining as was reported in an earlier paper (24). In the preparation of the detailed pen drawings illustrative of the foliage, flowering, and fruiting habit of representative evergreen and deciduous species (figs. 1–17), the author has had the professional assistance of Miss Stella Dernoga of the Art Institute, Baltimore.

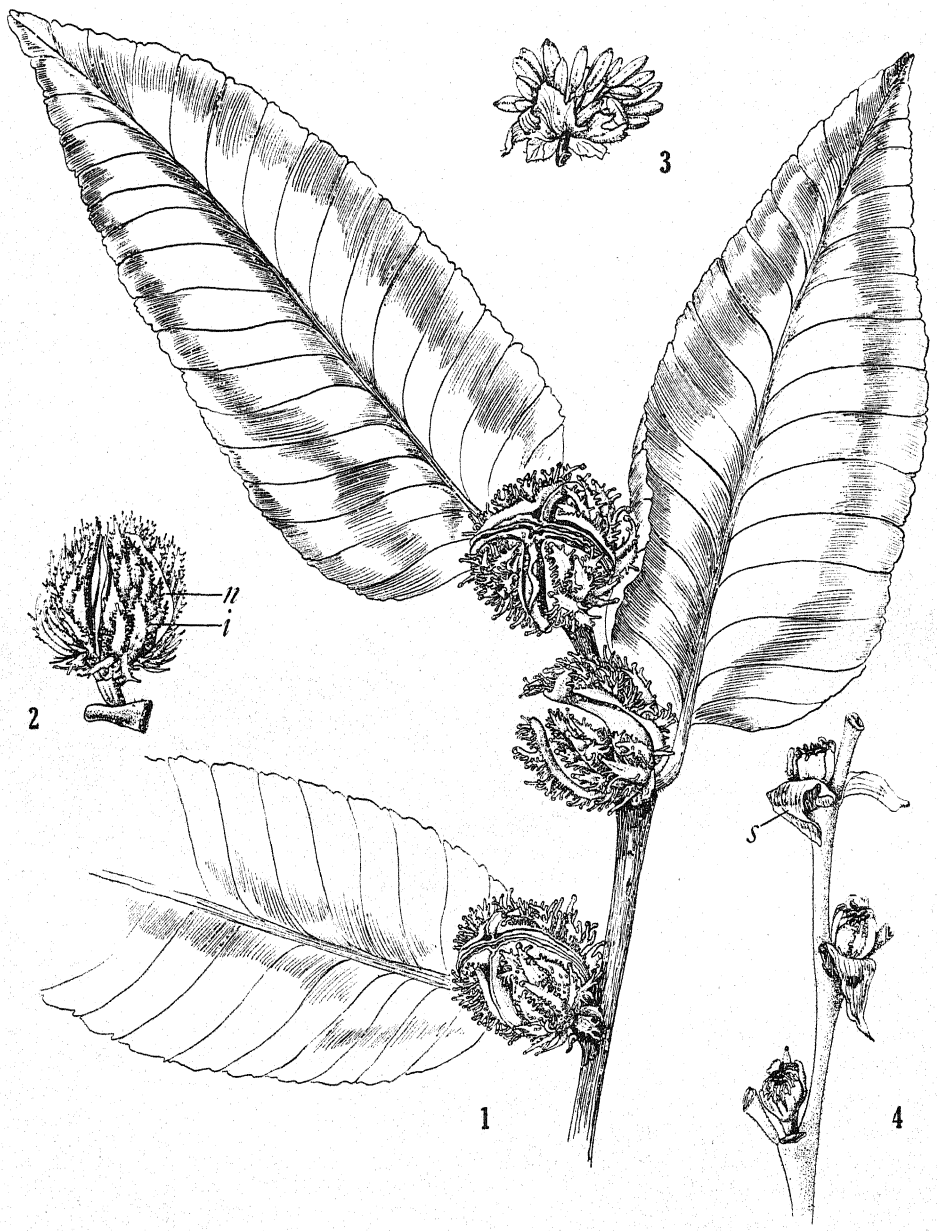
[4] Duplicate material of the New Guinea beeches was sent to Dr. A. Pulle, University of Utrecht, for study, from the Arnold Arboretum, prior to the outbreak of the war. Although to date no report has been received from Pulle, recent reports indicate that one of the New Guinean species of *Nothofagus* was published by Japanese botanists in Java during the war years and incorrectly classified as a new genus of the Hamamelidaceae.

Morphological characters

Various natural systems of classification of the Fagaceae or Cupuliferae have been proposed. In most of them the beeches of both northern and southern hemispheres are segregated as a natural group or subfamily, the inflorescences of which are described as simple one- to many-flowered dichasia in the axils of the leaves; the male flowers either in dense slender-stalked clusters or in short one- to three-flowered cymes; the female (one, two, or three in number) incompletely inclosed by a two- to four-lobed bracteolate involucre, e.g., the Fagineae of Oerstedt (28) and the Fageae of Prantl (30). Two genera are recognized—*Fagus* and *Nothofagus*.

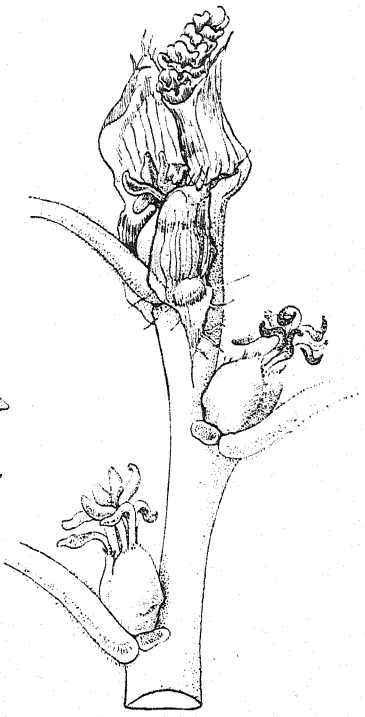
Nothofagus comprises those monoecious, occasionally dioecious, shrublike or arborescent species, with simple two-ranked, generally very small leaves; some species are deciduous, others evergreen in habit.

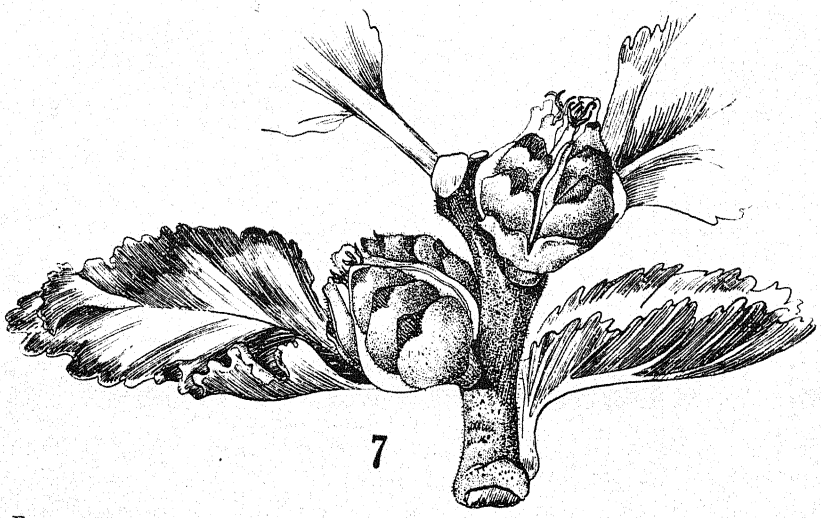
Leaf characters.—In the six deciduous species, all South American with the exception of the Tasmanian species *N. gunnii*, the leaves (1.5–6.5 cm. long and 1–3 cm. wide) are soft in texture, generally folded in the bud along the lateral nerves (fig. 5), and either crenate or serrate in margin, with variable degrees and types of serration in the different species. The large multicellular dermal glands, so conspicuously identified with the leaves of the evergreen beeches, may or may not occur in association with the leaves of the deciduous species. In *N. antarctica* (fig. 5), as in *N. procera* and *N. pumilio*, resinous papillae are distributed on both



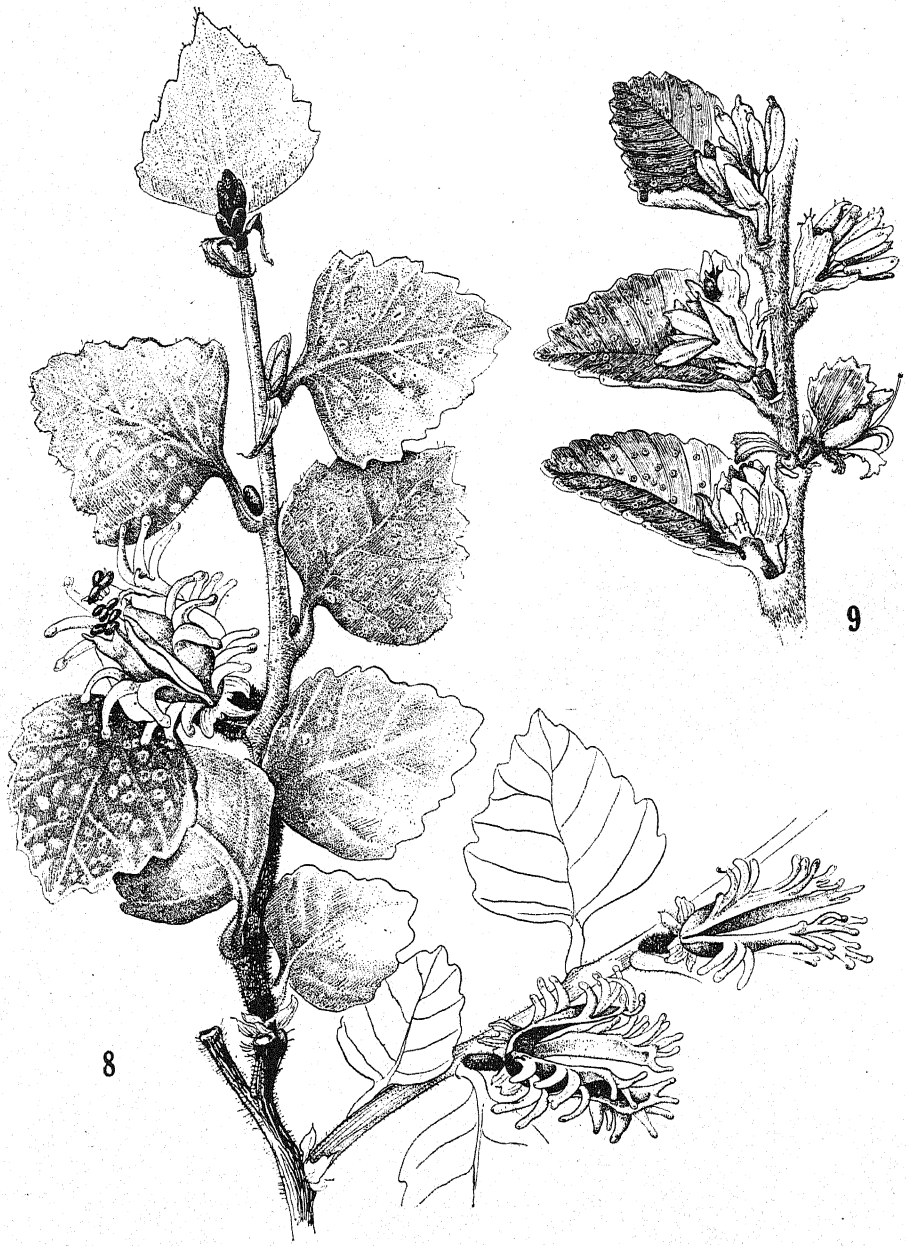
FIGS. 1–4.—*Nothofagus procera*, Chile. Fig. 1, fruiting habit; nutlets, three in number, closely invested by four-valved cupule; valves with leafy pinnatifid appendages bearing stalked glands. ×1.7. Fig. 2, young fruiting head; *n*, wing of nutlet; *i*, segment of lobular cupule. ×1.5. Fig. 3, single staminate floret. ×2. Staminate flowers in this species may be borne singly or in groups of three. Fig. 4, three pistillate inflorescences, upper two with associated stipules (*s*). ×1.5.



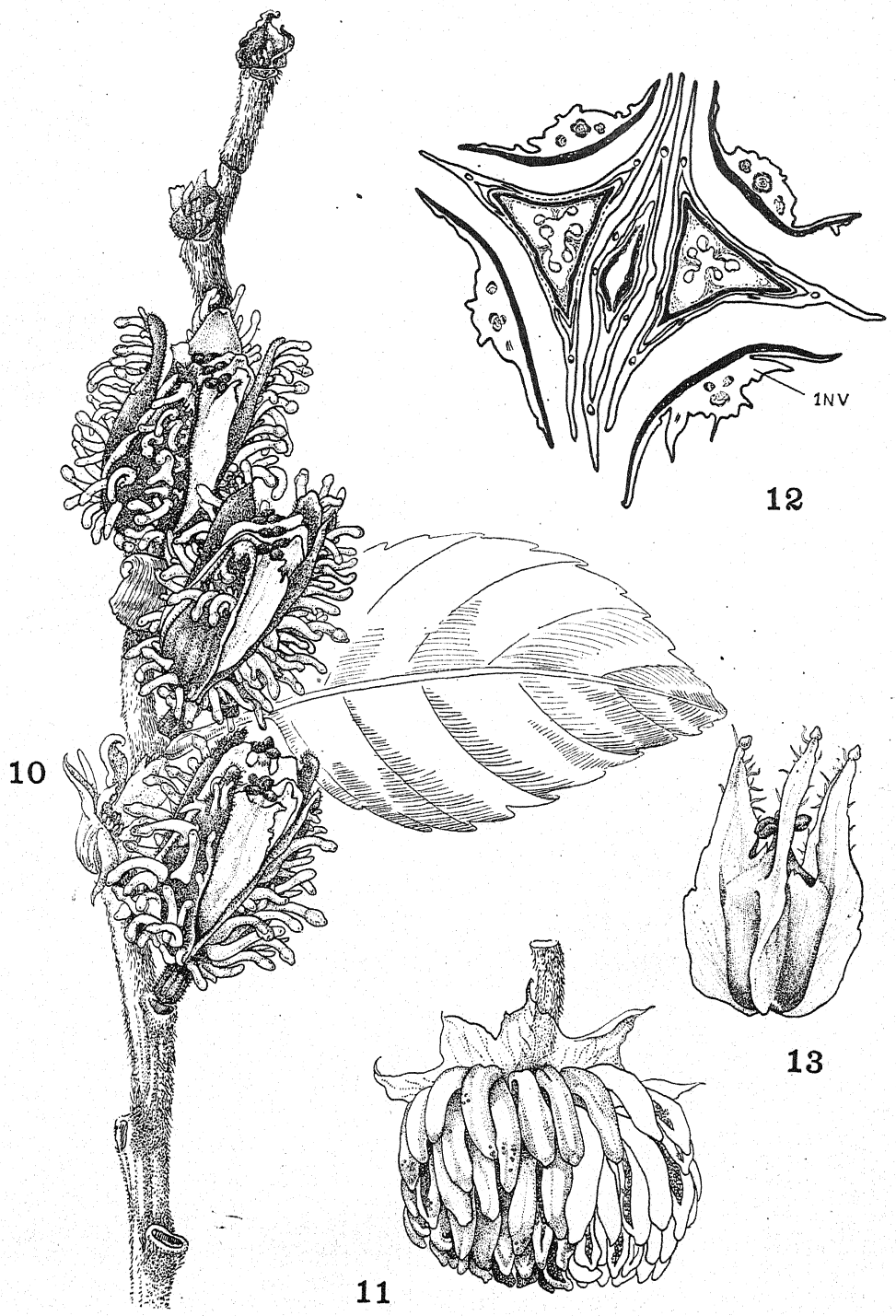




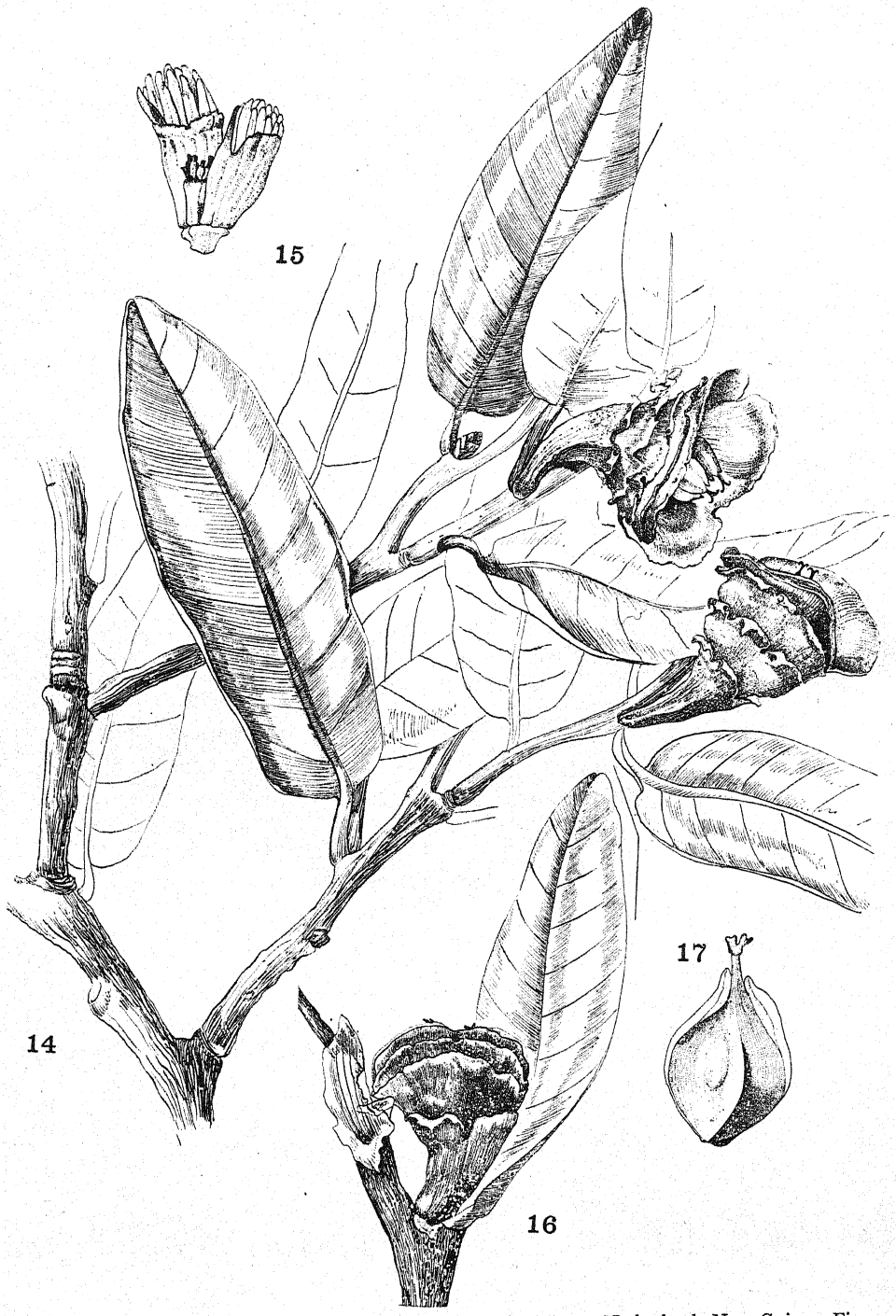
FIGS. 5–7.—*Nothofagus antarctica*, Chile and Argentina. Fig. 5, foliage and flowering habit (staminate flowering group). ×2.6. Fig. 6, pistillate inflorescences, upper group with associated stipules. ×4.6. Fig. 7, cupule-inclosed fruits. Cupule inclosing nutlets is four-valved, each valve with three to five very short transverse scarious scales. ×2.6.



FIGS. 8–9.—*Nothofagus cunninghami*, Tasmania. Fig. 8, foliage and fruiting characters, in particular conspicuously glandular character of both foliage leaves and involucral scales. ×2.6. Fig. 9, flowering shoot bearing both staminate and mixed inflorescences, also solitary pistillate floret. ×2.6.



FIGS. 10–13.—Figs. 10, 11, *Nothofagus moorei*, Australia. Fig. 10, fruiting habit. ×3. Fig. 11, single staminate floret, showing characteristic form and mode of dehiscence of anthers in *Nothofagus*. ×3. Figs. 12, 13, *Nothofagus menziesii*, New Zealand. Fig. 12, transection of nutlets with inclosing cupule valves; shaded areas indicate distribution of sclerotic tissue within pericarp and cupular valves. Fig. 13, single nutlet, illustrating characteristic winged character of fruits in *Nothofagus;* wings of this species project well above stigma lobes and are gland-tipped. ×4.6.

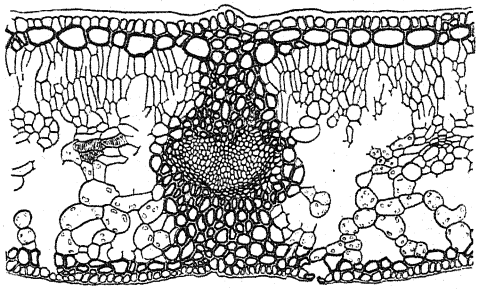


FIGS. 14–17.—*Nothofagus* (BRASS and VERSTEEGH 11115) sp. nov., Netherlands New Guinea. Fig. 14, foliage and fruiting habit; fruiting heads consist of woody bivalved funnel-form cupule which invests three nutlets (two are three-winged; one is two-winged). Lobes of cupule joined almost to rim, each lobe covered externally by three or four flat lobular scales. ×1.3. Fig. 15, staminate inflorescence (immature, with one floret aborting). ×2.6. Fig. 16, young fruiting head, fruits still closely invested by involucral segments. ×2.6. Fig. 17, single nutlet; winged character of fruits not so prominent as in antarctic members of genus.

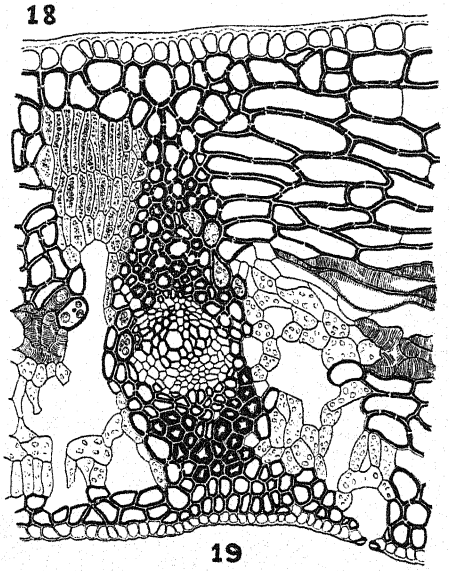
the adaxial (upper) and the abaxial (lower) surfaces of the leaves, usually in close association with the veins. In *N. obliqua*, as in *N. montagnei*, they occur infrequently and only on the upper sides of the leaves.

In the evergreen species, predominantly found in Australia and New Zealand with the exception of the New Guinea species and the two species in South America, the leaves are usually coriaceous and glabrous or with variable pubescence, the margins either entire or minutely crenulate, and practically all dotted with resinous papillae on either the upper or the lower surfaces, occasionally on both. In *N. cunninghami*, glands are borne only at the margins and on the adaxial faces of the small leathery leaves (figs. 8, 9); in *N. menziesii*, glands are sparingly distributed only on the abaxial surfaces; whereas, the equally small, thick leaves of *N. dombeyi* and those of *N. betuloides* are glandulous punctate on both lower and upper surfaces. With the exception of the Australian species, *N. moorei* Krasser, and the recently discovered beeches of the New Guinea area, the evergreen species are characterized by exceedingly small leaves (e.g., 1.2–1.5 cm. in *N. cliffortioides*, 1.5–2 cm. in *N. betuloides*, and 0.5–1.5 cm. in *N. cunninghami* and *N. menziesii*). The leaves of the larger-leaved species of evergreen beech average 2–4 cm. long, occasionally 8–10 cm. on the younger vegetative shoots, and are sharply serrate in their margins. The thick leathery leaves of the New Guinea beeches range 2.5–5.0 cm. long and 1.4–2.0 cm. wide and are characterized by entire, conspicuously recurved margins, with a distinctive notching of the apical portion (figs. 14, 16).

The histological structure of the leaves of these beeches of the antarctic may be described as markedly xeromorphic in most of the evergreen species and as quite typically mesomorphic in the deciduous species, except for a tendency to lignification of both the epidermal and the hypodermal layers and a prominent development of sclerotic tissues in association with the veins. Among



18



19

FIGS. 18–19.—Fig. 18, *Nothofagus cunninghami*, transection of midrib portion of foliage leaf, with adjacent tissues. ×100. Fig. 19, *Nothofagus* (BRASS and VERSTEEGH 11115) sp. nov., transection of leaf showing one of smaller lateral veins and adjacent tissues. ×110.

the evergreen forms, possibly as an adaptation to the climatic extremes of their environment, all gradations occur in the degree of cuticularization—from the formation of cuticle layers 0.05–0.1 mm. thick, as in *N. cunninghami* (fig. 18), to the elaborate thickenings in the leaves of

certain of the New Guinea species (fig. 19) and in *N. cliffortioides*.

Along with well-developed cutin layers, these evergreen beeches exhibit different degrees of lignification of the cells of the epidermal, as do also those of the hypodermal, layers. In *N. solandri*, and in certain of the smaller-leaved New Guinea beeches (e.g., 11335 of the BRASS and VERSTEEGH collection), there is no marked thickening of either epidermal or hypodermal cells except in the vicinity of the midrib and lateral veins. In *N. cunninghami* the epidermal and hypodermal cells on both sides of the leaf have thick walls, particularly those of the adaxial side. In certain of the larger-leaved New Guinea beeches (e.g., BRASS and VERSTEEGH 11997*a*, 11115, and 10479), the thick-walled hypodermal region is several cells in depth, on both the adaxial and the abaxial sides, and ribs of sclerenchyma tissue extending from the upper and lower hypodermal layers completely envelop the lateral veins, along with the vascular tissues of the midrib, in heavily sclerotinized sheaths. Masses of irregularly shaped scalariform-perforated elements not uncommonly are to be found in such cases in the parenchyma areas between or immediately adjacent to the sclerenchyma-sheathed veins (figs. 18, 19). It is perhaps significant—certainly noteworthy—that among morphological characters emphasized in the Lyginopterideae (36) have been the marked development of sclerenchyma, in the form both of hypodermal ribs and of sclerotic plates located within the internal tissues of stems and leaves; likewise the conspicuous development of stalked, capitate glands in association with the leaf surfaces.

The palisade parenchyma is typically xeromorphic in the evergreen beeches, generally consisting of two to three subhypodermal layers, the cells of which are extremely small and narrow, many containing abundant resin deposits (fig. 19).

STAMINATE INFLORESCENCE AND FLOWER.—The male flowers, solitary or in groups of two to three on short recurved peduncles, in the monoecious species are borne either toward the base of the season's growth or on branches adjacent to those bearing the pistillate inflorescences. Their calyces are either campanulate or funnel form and multilobed. Solitary staminate florets rather than groups predominate in *N. menziesii*, *N. moorei*, *N. betuloides*, and *N. obliqua*, while in *N. dombeyi* and *N. nitida* the grouped arrangement of male florets appears to be a constant feature. In all other species of the genus, however, including the New Guinea beeches, the staminate flowers may occur either singly or in two- to three-flowered dichasia on the same plant.

It has been the writer's observation that the number of staminate flowers (one, two, or three), as also the stamen number, varying from eight to forty (eight to twelve in the smaller florets and twenty to forty in the larger solitary flowers), are characters hardly to be depended upon in the differentiation of the species. On the other hand, even though variable in size, the general form and structure of the anthers (figs. 3, 5, 9, 11, 15), as also the pollen characters, may be relied upon as distinctive of the genus—they are unlike the anther and pollen characters of other genera of the Fagaceae. In general, the anthers in *Nothofagus* conform to a linear-oblong pattern, are two-celled, and are distinctly apiculate; those of *N. procera* and *N. cunninghami* are shortly oblong, the latter with a distinctive curvature of the apex.

In but one of the several species of *Nothofagus* studied in detail has a tend-

ency to hermaphroditism in floral structure been observed, and that is in *N. cunninghami*, the irregularities of which are described under "Pistillate inflorescence and flower."

PISTILLATE INFLORESCENCE AND FLOWER.—The female flowers also occur in simple cymes—one, two, or three (typically three) in a head—the flower heads being borne on erect short peduncles usually in the upper axils of the season's growth, each flower cluster closely invested by a two-, three-, or four-lobed lamellate involucre and a complement of bracts corresponding to the secondary and tertiary floral units of a compound cyme.

Although the segregation of pistillate and staminate flowers in different inflorescences—the pistillate in the upper axils, the staminate in the lower axils of the season's growth—is the general rule in species of this genus, checked in detail in *N. obliqua*, *N. procera*, *N. antarctica*, *N. menziesii*, and *N. moorei*, a certain amount of variability in arrangement and composition of the flowering clusters has been observed, in particular in the Tasmanian species, *N. cunninghami*. The following inflorescence types have been observed in this form—all in association with the flowering shoots of a single season's growth: (*a*) normal three-flowered pistillate inflorescences; (*b*) normal three-flowered staminate inflorescences; (*c*) three-flowered inflorescences consisting of a center pistillate floret with staminate laterals, the latter on short peduncles; (*d*) three-flowered staminate heads, the center floret abortive; and (*e*) solitary pistillate florets, usually with a loose, scaly involucre and occasionally exhibiting abortive stamens (see fig. 9 for habit illustration of some of these types).

Whatever their position and organization, these small, generally inconspicuous, pistillate cymules are, during their early development, very effectually concealed by the broad, variously modeled, membraneous stipules of the subtending foliage leaf (figs. 4, 6).

A valvate or lobular involucre in association with the pistillate flowers and the nutlets is one of the distinctive features of both *Fagus* and *Nothofagus*, but in its external morphology it is extremely variable in the beeches of the southern hemisphere. Variability is seen in (*a*) the number of involucral valves; (*b*) the size and shape of the valves; (*c*) the character and number of the foliar appendages borne dorsally or marginally by the segments; and (*d*) the size and general character of the glands.[5] With the exception of the South American species *N. pumilio* and the evergreen beeches of New Guinea, the cupules associated with the pistillate flowers in species of *Nothofagus* are of the three- to four-valved type, the valves incompletely inclosing three flowers (one dimerous and two trimerous). The pistillate inflorescences of *N. pumilio* and of the New Guinea representatives, however, are characterized by a bivalved involucre which in *N. pumilio* incloses a

[5] The capitate glands, one of the particularly noteworthy and conspicuous features of the cupules of *Nothofagus*, especially of the antarctic species, are large multicellular structures, which, when not apparent as globular stalked appendages borne at the tips of the pinnatifid involucral scales (e.g., *N. moorei*, *N. menziesii*, *N. cunninghami*, and *N. procera*), are frequently to be disclosed in vertical sections of the pistillate inflorescences as sessile, conelike structures borne in the axils of the involucral leaves (fig. 26*A*) or in the axils of the bracts of the inflorescence or even the stipules.

The stalked variety of gland—bearing a striking resemblance to the "glandular spines" identified with the petioles and cupules of *Lyginopteris* (36)—though variable in size, are frequently massive organs (0.5–2.0 mm.), the stalked portion of which has the morphological features of a modified leaf segment. The capitate glandular portion of this organ (fig. 27*A*) consists of a layer of columnar secreting cells, apparently epidermal in origin, which surrounds a spongy core.

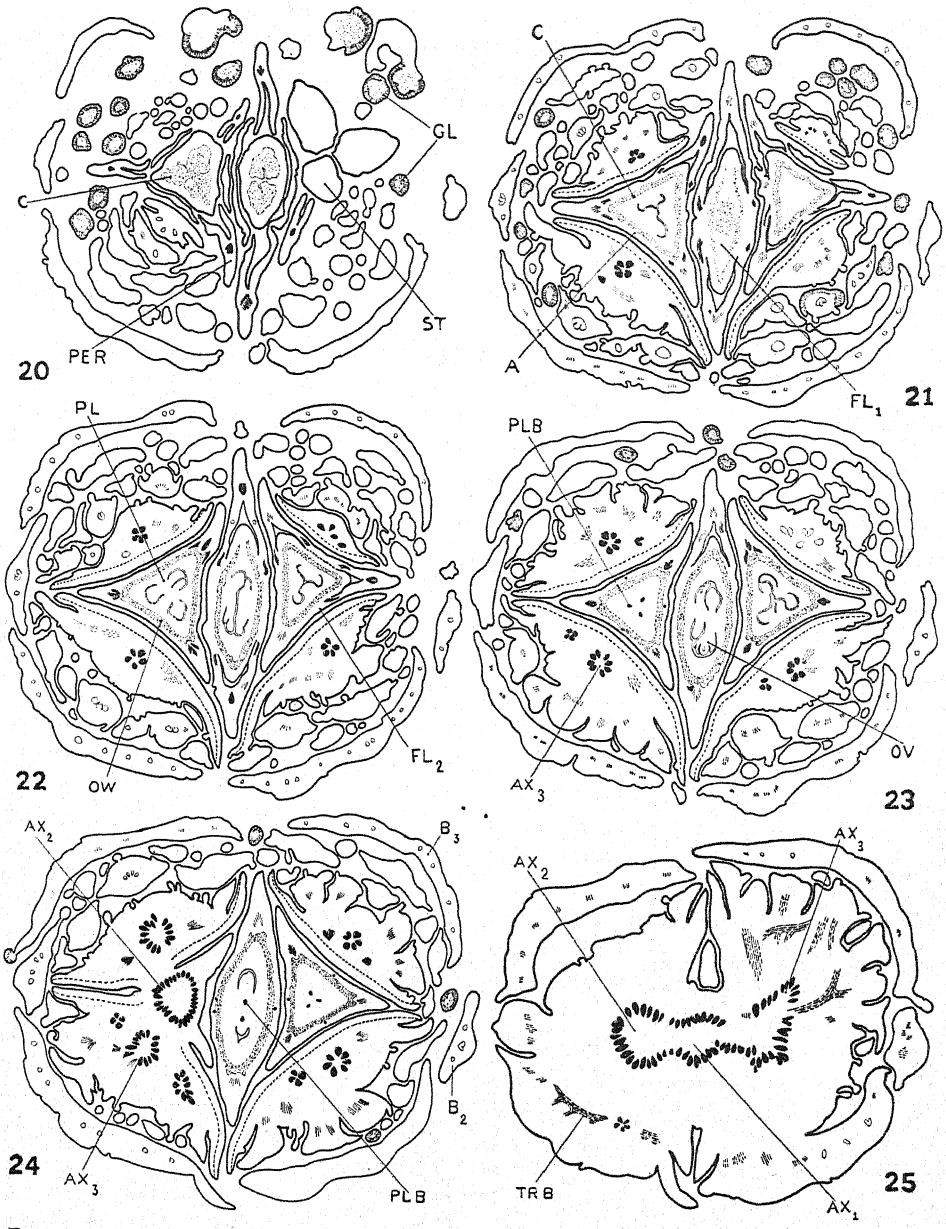
solitary floret and which, in the New Guinea species, incloses either a solitary dimerous floret or a two- to three-flowered group. In the case of the three-flowered groups the flowers may be associated with a single dimerous central floret with two trimerous laterals, or all three may be of the flattened dimerous type, each oriented in the plane of the involucral segments.

Notwithstanding the very noticeable variation in the sizes and shapes of the pistillate florets in the different species of the genus, and the character of their stigmas and conspicuous winged appendages (figs. 1, 6, 7, 8, 10, 13, 17), little variability among the species is apparent in the basic morphology of the ovary, in the origin of the ovules, or in details of vascularization of either carpels or ovules. The individual female flower consists normally of a four- to six-membered perianth (generally four sepals in the dimerous florets and six in the trimerous), borne on the rim of a fleshy and conspicuously angular bi- or tricarpellary ovary, the latter to be interpreted on the basis of ontogenetic evidence as receptacular tissue lined and concrescent with a carpellary core. The outer axial portion of the ovary wall is clearly distinguishable from that which is carpellate in origin, the former being characterized by a loose, somewhat fibrous, texture and the possession of vascular elements, whereas the inner carpellate section is distinguished by a marked zonation which includes an outer *woody* band of variable thickness (figs. 12, 22–25) and an inner fleshy zone. It is from the latter that the three two-ovuled placentae are derived.

Although an axile column of vascularized tissue develops concurrently with the placental tissue and, as in other genera of the Fagaceae (**24, 40**), eventually fuses with it, the relationship of this axile column to the placentae and ovules is not the same as that reported in *Fagus* and *Quercus* (**24**) or in *Castanopsis* (**6**), in all three of which the floral axis produces a thick columnar structure—well supplied with vascular tissue—at the tip of which ovule development is initiated.

VASCULAR ORGANIZATION OF PISTILLATE INFLORESCENCE.—The manner of distribution of the vascular tissues to the female flowers, and to the associated involucre and complex of bracts, is interesting and sheds considerable light on the highly controversial question of the morphological nature of the special involucre in the Fagaceae. A number of hypotheses, based upon studies of *Quercus*, *Fagus*, *Castanea*, and *Castanopsis*, have been proposed in explanation of this structure. Some emphasize the bracteolate character of the cupule (**16**), while others accentuate its essentially axial nature, either as an intercalary formation of the inflorescence appearing between the flower and its bracteoles (**29**) or as the product of the fused secondary or tertiary branches of a dichasium (**13, 26**).

In the vascular organization of the typical pistillate inflorescences in *Nothofagus* (i.e., three-flowered with a four-lobate involucre, well illustrated in *N. procera* and in *N. antarctica*), the three female flowers, one median and two laterals, receive their vascular supply, respectively, from the primary and the secondary vascular axes of the cymule. The vascular cylinder of each floret consists of fifteen to eighteen bundles, some distributed along the sides of the angular floral axis, with others closely associated at the ridged corners of the axis. Generally from the corner units of the floral vascular axis, strands to the central column of the ovary diverge at the base of the flower. Within the axile column of the lateral tricarpellate flowers these



FIGS. 20–25.—*Nothofagus procera*, successive transections of pistillate inflorescence at intervals from apical to basal portions. ×20. (*A*, axial part of ovary wall; AX_1, vascular axis of primary flower of inflorescence; AX_2, vascular axis of secondary floret; AX_3, vascular axis of modified tertiary branch; B_2, secondary bract; B_3, tertiary bract; *C*, carpellate section of ovary wall; FL_1, primary floret; FL_2, secondary floret; *GL*, gland; *OV*, ovule; *OW*, ovary wall; *PER*, perianth; *PL*, placenta; *PLB*, vascular supply to placentae; *ST*, stigma lobe; *TRB*, traces of tertiary bract.)

bundles form a three-strand converging vascular unit which eventually resolves itself into the double bundles supplying the funicles of the six ovules (figs. 23, 24). In the flattened median or primary floret of the typical three-flowered pistillate inflorescence, only two vascular units are observed in the placental axis (fig. 24). This two- or three-strand vascular supply of the axile column of the pistillate florets of *Nothofagus* may perhaps be compared with the inner placental bundles reported in *Castanopsis* (6). The prominent cylinder of vascular tissue associated with the axile column of the ovaries of the Castaneae, and with that of *Fagus* and *Quercus*, has not been detected in either the dimerous or the trimerous florets of *Nothofagus*.

The vascular elements to the inner perianth leaves and wings, and likewise those to the styles, are seen to proceed, as conjoint bundles, from the corner units of the floral vascular axis through the marginal (axial) part of the ovary wall to the point of their divergence either to wing, perianth, or stylar members. The rather weak vascular supply to the outer perianth leaves appears to be derived from certain of the units of the floral vascular axis which alternate with those supplying the inner sepals and styles.

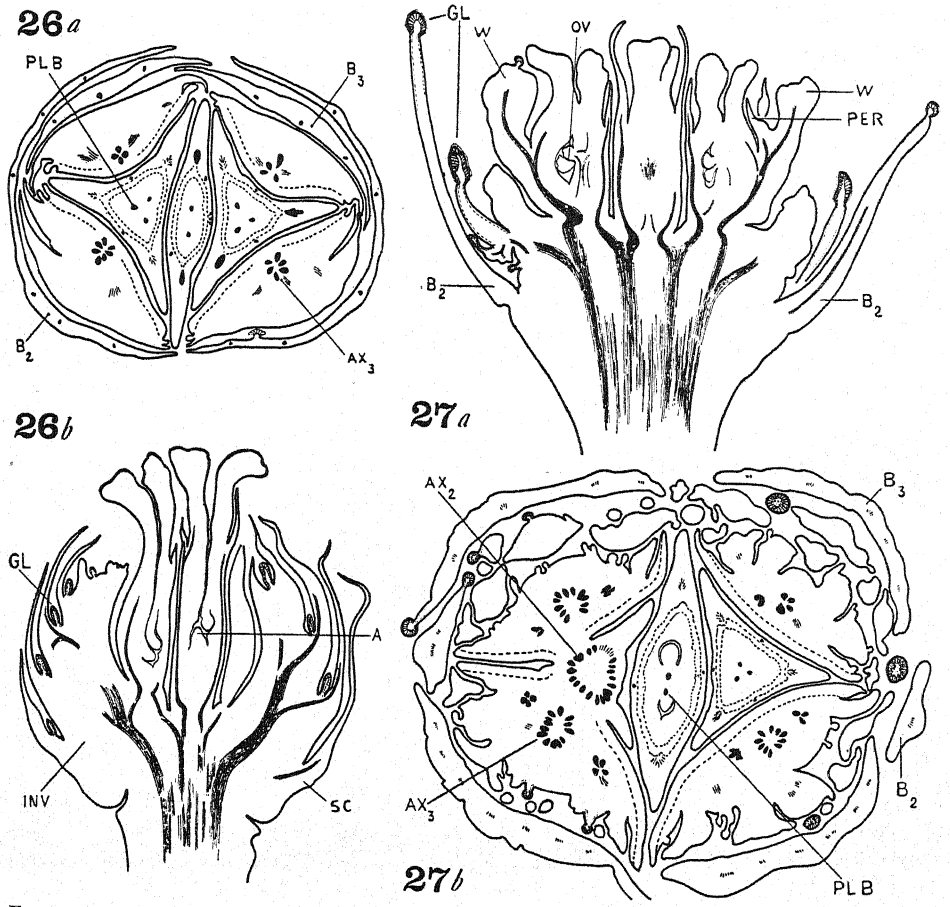
The involucral segments, extending to either side and at right angles to the lateral or secondary floral units of the dichasium, are largely axial in nature; each wedge-shaped valve bears in acropetal succession several to many either lobed or pinnatifid appendages, usually conspicuously glandular (figs. 26, 27). One of the distinctive features of the fruiting heads of the antarctic species of *Nothofagus* is the markedly woody nature of the inner section of the cupule valves, where they adjoin the nutlets (fig. 12), a feature not observed in the more specialized New Guinea species (figs. 28–33, 34–37) or as one of the involucral characters of *Fagus* (24). The numerous vascular bundles of each segment of a four-valved involucre will be seen to converge within the axial section as three groups, the center one of each group forming a well-defined, several-strand vascular cylinder, as in the vascular axes of the pedicels of the inflorescence. These "tertiary" pedicellar vascular units in conjunction with those of the secondary axes form a vascular cylinder oriented transversely to that of the combined primary and secondary axes of the inflorescence. The relationship between the vascular units of the involucral segments and those of the secondary axes of the inflorescence is clearly indicated in figures 20–25, depicting successive transections of the triflorous pistillate inflorescence of *N. procera*.

The vascular supply to each of the four tertiary bracts is, like that of the modified tertiary axes, derived from the vascular cylinders of the secondary axes of the dichasium, whereas that of the secondary bracts is derived from the vascular cylinder of the primary axis of the cymule.

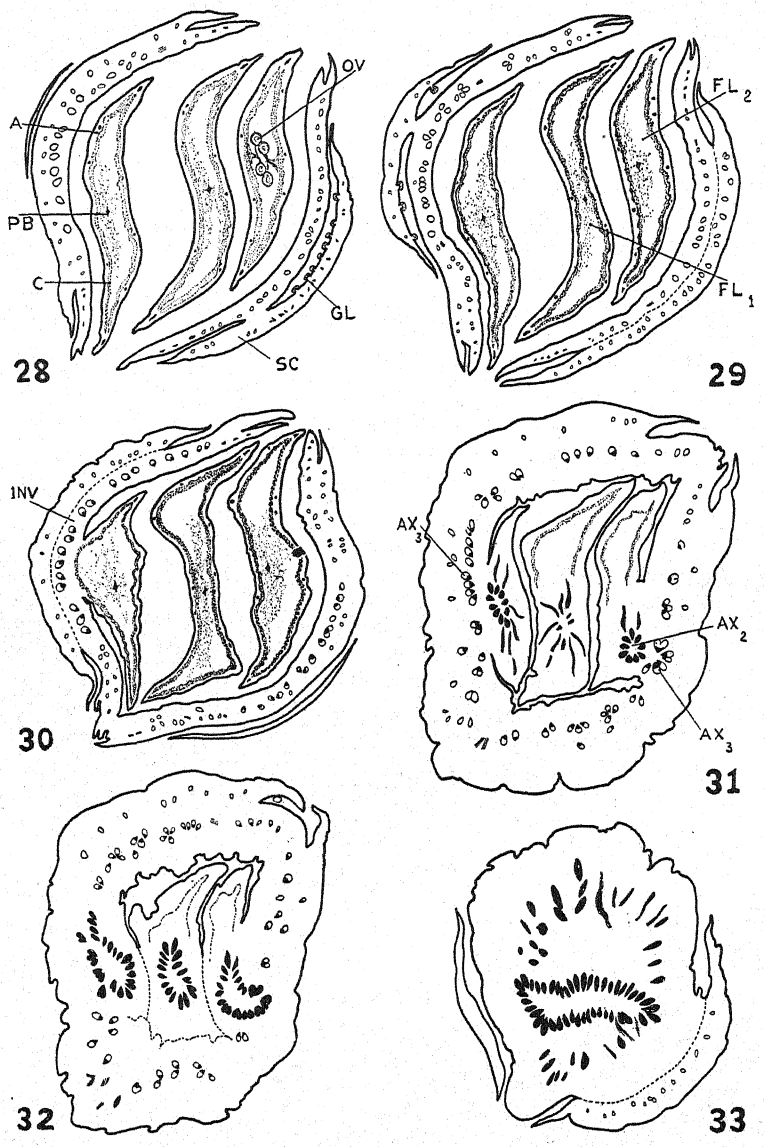
The vascular strands to the secondary bracts are distinct in every way from those directed to the lamina of the subtending foliage leaf and its stipules. Serial transverse sections made to the very base of the primary axis of the inflorescence reveal the vascular systems of each of the rather prominent stipules as very definitely related to the lateral components of the trilacunar trace system supplying the lamina of the foliage leaf which subtends the pistillate flowering group. This is very nearly in agreement with ABBE's interpretation of the vascularization of the secondary bracts and with that of the reduced foliage leaf sub-

tending the pistillate cymules in certain of the Betulaceae (1).

On the basis of vascular organization, therefore, the four bracteolate involucral valves of *Nothofagus* may be interpreted as corresponding to the bracts and modified floral parts, including the axial portions, of the "tertiary units" of an originally more complex cymose inflorescence. This idea has been suggested in a previous report by the writer (25) and has also been proposed, but not very convincingly supported, by BERRIDGE in her studies of the floral morphology of *Castanopsis chrysophylla* (6), in which she asserted that all evidence brought forward in previous work on *Fagus* and *Castanea*, as well as her own studies of *Castanopsis*, are applicable in support of the theory that the valves of the cupule of the Fagaceae are modified secondary or tertiary axes of a dichasial inflorescence and



FIGS. 26–27.—Fig. 26*A*, *B*, *Nothofagus antarctica*, transverse and median-vertical sections of pistillate inflorescence. ×17.5. Fig. 27*A*, *B*, *Nothofagus procera*, median-vertical and transverse sections of pistillate inflorescence. ×15. (*A*, axile column; AX_2, vascular cylinder of secondary axis; AX_3, vascular axis of modified tertiary branch; B_2, secondary bract; B_3, tertiary bract; *GL*, glands; *INV*, involucre; *OV*, ovule; *PER*, perianth; *PLB*, placental bundles; *SC*, involucral scale; *W*, winged extension of ovary wall. Dotted lines indicate areas of ovary wall and involucral valves which undergo sclerotinization during maturation of fruits.)



FIGS. 28–33.—*Nothofagus* (BRASS and VERSTEEGH 11363) sp. nov., Netherlands New Guinea, successive transections of triflorous pistillate inflorescence characteristic of species, all florets of which are flattened and bicarpellate. ×6. (*A*, axial part of ovary wall; AX_2, vascular axis of secondary floret; AX_3, displaced bundles representing vascular units of modified tertiary axes; *C*, carpellate section of ovary wall; FL_1, primary floret; FL_2, secondary floret; *INV*, one of segments of bivalvate involucre; *GL*, gland; *OV*, ovule; *PB*, placental bundles; *SC*, involucral leaves.)

not, as EICHLER (16) maintained, merely bracts covered with emergences.

Further evidence in support of this hypothesis is supplied by the single-flowered, and the reduced three-flowered, pistillate inflorescences distinguishing the New Guinea species of *Nothofagus*, although the interpretation of the cupule morphology of these beeches would indeed be difficult without some understanding of the inflorescence morphology of less specialized members of the genus.

COMPARISON OF NEW GUINEA SPECIES WITH OTHER SPECIES OF NOTHOFAGUS

Taxonomic studies dealing with the beeches of the southern hemisphere (17, 28, 32) have been in general agreement in emphasizing leaf and involucral characters in keys to the genus, and on the basis especially of foliage characters the sixteen recognized species have been referred to one of three natural sections:

I. Leaves *deciduous*, soft in texture and folded in the bud along the lateral nerves, margins either crenate or serrate; the species in this section, with the exception of *N. pumilio*, all distinguished by four-merous involucres generally with dorsal glands. (1) *N. antarctica* Oerst.; (2) *N. montagnei* Reiche; (3) *N. pumilio* Krasser; (4) *N. obliqua* Blume; (5) *N. procera* Oerst.; (6) *N. gunnii* Oerst.

II. Leaves *evergreen*, usually very coriaceous and glabrous, or with only slight pubescence, margins coarsely or finely serrate or crenate, the surfaces of the leaves—either upper, lower, or both—conspicuously dotted with resinous papillae; the species of this section also characterized by four-merous involucres, the segments generally with leafy pinnatifid appendages with stalked glands. (7) *N. apiculata* Krasser; (8) *N. menziesii* Oerst.; (9) *N. cunninghami* Oerst.; (10) *N. betuloides* Blume; (11) *N. dombeyi* Blume; (12) *N. nitida* Krasser; (13) *N. fusca* Oerst.; (14) *N. moorei* Krasser.

III. Leaves *evergreen* with entire margins, the leaves on young trees generally glabrous, those on older trees densely tomentose; the two species in this section, (15) *N. cliffortioides* Oerst. and (16) *N. solandri* Oerst., both distinguished by three-merous involucres.

It is with the third section of the three that the New Guinea beeches appear to be most clearly allied, although they exhibit characteristics sufficiently distinctive in their leaves, cupules, and flowers to warrant their recognition either as a subsection of III or as an independent fourth section of the genus.

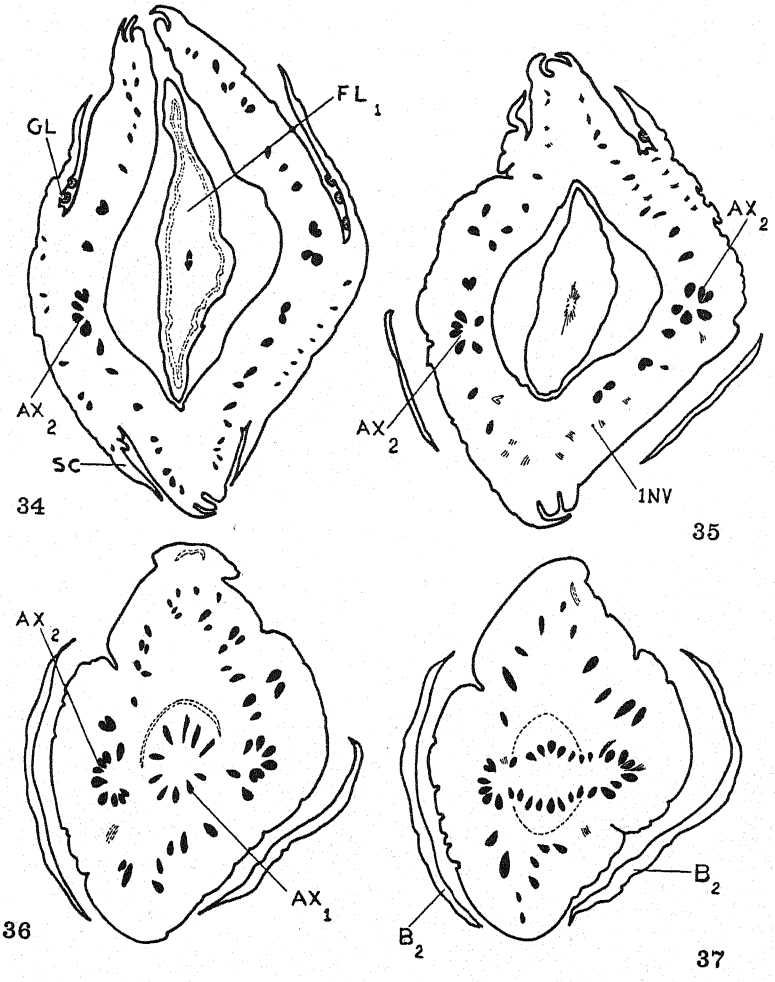
With one exception (i.e., BRASS and VERSTEEGH 11335), the five types of New Guinea beeches examined by the writer are characterized by *evergreen* leaves, thick and coriaceous in texture, their upper surfaces glabrous, the lower somewhat tomentose and conspicuously dotted with resinous papillae, margins entire and recurved with a distinctive notching of the apical section. The sizes are variable, ± 3.5–4.5 cm. in length by ± 1.5–2.0 cm. in breadth, but generally are larger than those of the antarctic members of the genus. As in other species of *Nothofagus* broad, scalelike stipules, peltate in shape and glandular at the base, are a conspicuous feature of the younger shoots, partially investing the young foliage leaves and the flower buds.

The anatomical features of the leaves of these beeches have already been described and compared with those of other evergreen species in the genus. Summarized, they include: (*a*) heavy cuticularization; (*b*) lignification of the cells of both the epidermal and the hypodermal layers, the latter frequently several layers in extent; (*c*) grouping of hypodermal cells into ribs in association with the vascular tissues; and (*d*) occurrence in the larger-leaved species of scalariform perforated transfusion elements bordering, or in the immediate vicinity of, the sclerenchyma-sheathed veins (fig. 19).

The four or five distinct species represented in this New Guinea collection are all characterized by a thick, scaly bi-

valvate cupule, the segments of which may be free almost to the base of the fruiting head or joined almost to the rim of the spreading valves (fig. 14). This funnel-formed cupule may inclose either a solitary flattened and two-winged nutlet (e.g., BRASS and VERSTEEGH 12673, 11333), illustrated in section in figures 34–37, or a group of three, in which the nutlets are associated, two trigonous and three-winged with one flattened and two-winged (e.g., BRASS and VERSTEEGH 11115, 11369), or all three may be of the flattened two-winged type and all oriented diagonally to the involucral lobes (e.g., BRASS and VERSTEEGH 11363), illustrated in section in figures 28–33.

In their bilobed involucres and predominantly bicarpellate dimerous florets, the latter frequently reduced to a solitary flower per cymule, the New Guinea beeches exhibit greater evidence of specialization than is apparent in the more typical members of the genus, i.e.,



FIGS. 34–37.—*Nothofagus* (BRASS and VERSTEEGH 11335) sp. nov., Netherlands New Guinea, successive transections of uniflorous pistillate inflorescence characteristic of species. ×13. AX_1, vascular cylinder of primary floral axis; AX_2, vascular units of modified secondary axes; B_2, secondary bract; FL_1, primary floret of cymule; *GL*, gland; *INV*, one of segments of bilobed cupule; *SC*, involucral leaves.)

those native to antarctic South America and to the Australian–New Zealand area. The latter, in their predominantly small evergreen leaves and tricarpellate florets —the pistillate flowers and the fruits associated usually in groups of three within a woody and conspicuously glandular four-valved involucre—exhibit a combination of characters which, in the opinion of the writer, is the most primitive to be found in the existing members of the beech series, if not in the entire Fagacean assemblage.

The morphological features of the flattened dimerous floral types, predominating in the New Guinea beeches, are about as we find them in the median floret of the more typical triflorous inflorescences in the genus (figs. 20–25). The ovary wall is thinner, however, and lacks the woody tissue characterizing other species of *Nothofagus;* likewise, there is a noticeable absence of sclerotic tissue in the thick involucral valves. The multicellular resin-secreting glands, borne dorsally by the involucral leaves of most of the beeches of the southern hemisphere (figs. 1, 8, 10), are in the New Guinea species borne ventrally by the involucral scales (figs. 29, 34).

In the triflorous pistillate inflorescences the vascular supply of each of the broad semicircular involucral valves (figs. 28–33) is seen to consist of a rather loosely organized core of vascular tissue and, extending to either side of this, of numerous bundles which spread fanlike from the point of their divergence from the vascular cylinders of the secondary axes of the dichasium. These bundles, reinforced by the traces of the involucral leaves, may be interpreted as the displaced vascular elements of the highly modified tertiary branches of the inflorescence. Likewise, on the basis of vascular organization, it seems evident that the involucral valves of the single-flowered cymules in this group (figs. 34–37) bear the same relationship to the primary axis of the inflorescence that the secondary axes bear in the more typical three-flowered cymules. There can be little doubt, moreover, that each of the cupule segments of the bivalved uniflorous species of *Nothofagus* represents one of the greatly modified secondary axes of a dichasial inflorescence. In the New Guinea species of *Nothofagus*, reduction and general modification of an originally more complex dichasial inflorescence have extended not only to tertiary but in certain cases even to secondary units, the florets of which have either been lost or greatly modified in the process of reduction of the inflorescence.

The vascularization of the pistillate flowers, as does the manner of origin of placentae and ovules, follows the same basic plan as that of other species of the genus.

Summary

1. Anatomical features, not hitherto reported, found to characterize the foliar organs, expecially of the evergreen species of the genus *Nothofagus*, include: (*a*) heavy cuticularization; (*b*) lignification of the cells of both the epidermal and the hypodermal tissues, the latter occasionally several layers in extent; (*c*) grouping of hypodermal cells into ribs in association with the vascular tissues; and (*d*) occurrence in the larger-leaved species of scalariform-perforated transfusion elements bordering, or in the immediate vicinity of, the sclerenchyma-sheathed veins.

2. Internal morphological characters in the flowers and fruits, which have been determined to be sufficiently constant in nature to supplement external morphological characters in a definition of this genus, include: (*a*) the occurrence of generally heavy, though variable, amounts of sclerotinization both of the ovary wall

and of the cupular valves; (*b*) a distinctive type of origin and vascularization of the placentae; (*c*) the occurrence of distinctive multicellular resin-secreting glands in association with either the dorsal or the ventral surfaces of the involucral scales, the cupules of certain of the species—in their peculiarities of glandular structure—bearing a striking resemblance to the gland-bearing husks enveloping the seeds of the Lyginopterideae.

3. The anatomy and morphology of the flowers and inflorescences of species of this genus furnish evidence in support of (*a*) the origin of the basic triflorous inflorescence characterizing the Fageae from an ancestral many-flowered dichasium, the individual florets of this hypothetical cyme being trimerous and probably bisporangiate, and of (*b*) the evolution of the lobular type of cupule in association with the pistillate florets as the product of reduction and general modification, including fusion, of sections of this originally more complex cymose inflorescence, such modification accomplishing greater protection for a few of the more advantageously located florets and fruits.

4. The beeches from Netherlands New Guinea, concerning which there has been some question as to position in the Fagacean series, have been found to exhibit a combination of morphological characters in their foliage and reproductive structures which is distinctly that of *Nothofagus*, although in certain respects they are sufficiently unlike the more typical members of that genus to justify their reference to a new section of the evergreen beeches.

Grateful acknowledgment is made to Dr. ELMER D. MERRILL, Arnold Professor of Botany, Harvard University, for the generous loan of herbarium materials as well as for valuable suggestions in the progress of this work. The writer is also indebted to Dr. A. C. COTTON, Keeper of the Herbarium and Library, and to Dr. C. A. METCALFE, Keeper of the Jodrell Laboratory at Kew Gardens, London, for fixations of flowering specimens of *Nothofagus* species; likewise to the Regius Keeper of the Royal Botanic Garden of Edinburgh.

DEPARTMENT OF BIOLOGY
GOUCHER COLLEGE
BALTIMORE, MARYLAND

LITERATURE CITED

1. ABBE, E. C. Studies in the phylogeny of the Betulaceae. BOT. GAZ. **97**:1–67. 1935.
2. ———. Studies in the phylogeny of the Betulaceae. II. Extremes in the range of variation of floral and inflorescence morphology. BOT. GAZ. **99**:431–469. 1938.
3. Bailey, I. W. The relation of the leaf-trace to the formation of compound rays in the lower dicotyledons. Ann. Bot. **25**: 225-241. 1911.
4. BENSON, M. Contributions to the embryogeny of the Amentiferae. Part I. Trans. Linn. Soc. Bot. (London), 2d ser., **3**:409–424. 1894.
5. BENTHAM, A., and HOOKER, J. D. *Fagus*, section *Nothofagus*. Genera Plantarum **3**:410. 1880.
6. BERRIDGE, E. M. The structure of the flower of Fagaceae, and its bearing on the affinities of the group. Ann. Bot. **28**:509–526. 1914.
7. BERRY, E. W. Tree ancestors, a glimpse into the past. Baltimore. 1923.
8. BESSEY, C. E. The phylogeny and taxonomy of angiosperms. BOT. GAZ. **24**:145–178. 1897.
9. BLUME, C. L. *Nothofagus*. Mus. Bot. Lugd. Bat. **1**:307. 1850.
10. CABRERA, A. L. Las Compuestas del Parque Nacional del Nahuel Huapi. Rev. Museo La Plata (nueva ser.), Sec. Bot. **1**:230–233. Buenos Aires. 1936–1938.
11. CAIN, S. A. Foundations of plant geography. New York. 1944.
12. CAMUS, A. Les Chênes. Monographie du genre *Quercus* sous-genre Cyclobalanopsis: Sous-genre Euquercus, sections Cerris et Mesobalanus. Encycl. écon. sylviculture **6**. Paris. 1936–1938.
13. CELAKOVSKÝ, L. Über die Cupula und den

Cupularfruct-Knoten. Oesterr. Bot. Zeitschr. 24:358–370. 1874.
14. COCKAYNE, L. Monograph on the New Zealand beech forests. Wellington, N.Z. 1926.
15. DUSÉN, P. The vegetation of western Patagonia. Rept. Princeton Univ. Exped. Patagonia 8. 1903.
16. EICHLER, A. W. Blüthendiagramme. 1:20–30. 1875.
17. ELWES, H. J., and HENRY, A. The trees of Great Britain and Ireland. Vol. 3. Edinburgh. 1905.
18. ENGLER, A. Angiospermae. *In* ENGLER and PRANTL, Die natürlichen Pflanzenfamilien. (Aufl. 2.) Bd. 14*a*. 1926.
19. FISHER, M. J. The morphology and anatomy of the flowers of the Salicaceae. Amer. Jour. Bot. 15:307–326, 372–394. 1928.
20. HOOKER, SIR W. J. Notes botanical Antarctic voyage 64. 1843.
21. ———. Icones Plantarum. Vol. 3 (new ser.): Pls. 630–631 (*Fagus fusca* Hook. f.); 639 (*Fagus Solandri* Hook. f.); 652 (*F. Menziesii* Hook f.). 1844. Vol. 5 (new ser.): Pls. 816*b* (*Fagus cliffortioides* Hook. f.); 881 (*F. Gunnii* Hook. f.). H. Bailliere, London. 1852.
22. HUTCHINSON, J. The families of flowering plants. I. Dicotyledons. London. 1926.
23. KRASSER, F. Ann. K. K. naturhist. Hofmuseums (Wien) 11:149–163. 1896.
24. LANGDON, L. M. Ontogenetic and anatomical studies of the Fagaceae and Juglandaceae. BOT. GAZ. 101:301–327. 1939.
25. ———. Report of progress in studies of the Fagaceae. Year Book Amer. Phil. Soc. Pp. 158–159. 1941.
26. LENDNER, A. Sur la cupule des Fagacées Bull. Soc. Bot. Genève 8:161–166. 1916.
27. MANNING, W. E. The morphology of the flowers of the Juglandaceae. I. The inflorescence. Amer. Jour. Bot. 25:407–419. 1938.
28. OERSTEDT, A. S. Beitrag til Kundskab om Engefamilien. K. Danske vidensk. Selsk. Skrift. 5:331. 1873.
29. PRANTL, K. Beiträge zur Kenntniss der Cupuliferen. Bot. Jahr. Engler 8:321–336. 1878.
30. ———. Fagaceae. *In* ENGLER and PRANTI, Die natürlichen Pflanzenfamilien. Teil III, Abt. 1:47–58. 1889.
31. REICHE, C. H. Beiträge zur Kenntnis chilenischen Buchen. Valparaiso. 1897.
32. ———. Key to the Chilean species of *Nothofagus*. Verhand. Deutsch. Wissenschaft. Vereins (Santiago, Chile) 3 (3): 402–403. 1897.
33. RENDLE, A. B. Classification of the flowering plants. 2d ed. Vol. 1. Cambridge. 1930.
34. SCHACHT, H. Der Baum. 1st ed. Berlin. 1853.
35. SCHWARTZ, O. Entwurf zu einem natürlichen System der Cupuliferen und der Gattung *Quercus*. Not. Bot. Gart. Mus. Berlin-Dahlem. 13:1–22. 1936.
36. SCOTT, D. H. Studies in fossil botany. 3d ed. Vol. 2. 1923.
37. SINNOTT, E. W., and BAILEY, I. W. Investigations on the phylogeny of the angiosperms. 4. The origin and dispersal of herbaceous angiosperms. Ann. Bot. 28:547–600. 1914.
38. SOLEREDER, H. System Werth Holzstructure. Stuttgart. 1885.
39. SKOTTSBERG, C. Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande 1907–1909. Vol. 5. Stockholm. 1916.
40. TRELEASE, W. The American oaks. National Academy of Sciences, Memoir 20:1–225. 1924.
41. WULFF, E. V. Historical plant geography. Academy of Sciences USSR. P. 525. 1944.

MORPHOLOGY OF SPORE FORMS AND HETEROECISM OF THE GIANT BAMBOO RUST, DASTURELLA DIVINA

M. J. THIRUMALACHAR, M. J. NARASIMHAN, AND K. S. GOPALAKRISHNAN

Introduction

Leaf rust on giant bamboo (*Dendrocalamus strictus* Nees) has recently been decribed by MUNDKUR and KHESWALLA (6) as belonging to a new genus *Dasturella* and as a member of the tribe Raveneliae of DIETEL (4). It had previously been described by SYDOW (7) as *Angiospora divina*, based on collections made by Dr. R. N. TANDON at Majhgawan, India, the host being tentatively identified as *Bambusa* sp. In a re-examination of the material, MUNDKUR and KHESWALLA found that the telial heads, instead of being nonerumpent and lenticular as in the genus *Angiospora* Mains, were distinctly erumpent and formed flabelliform crusts. As the rust was thus distinct from any species of *Angiospora*, they proposed the new genus *Dasturella*

for its accommodation. The host was reidentified as *Dendrocalamus strictus*. *Dasturella divina*, which is the type of the genus, has been recorded from Tonkin and Japan.

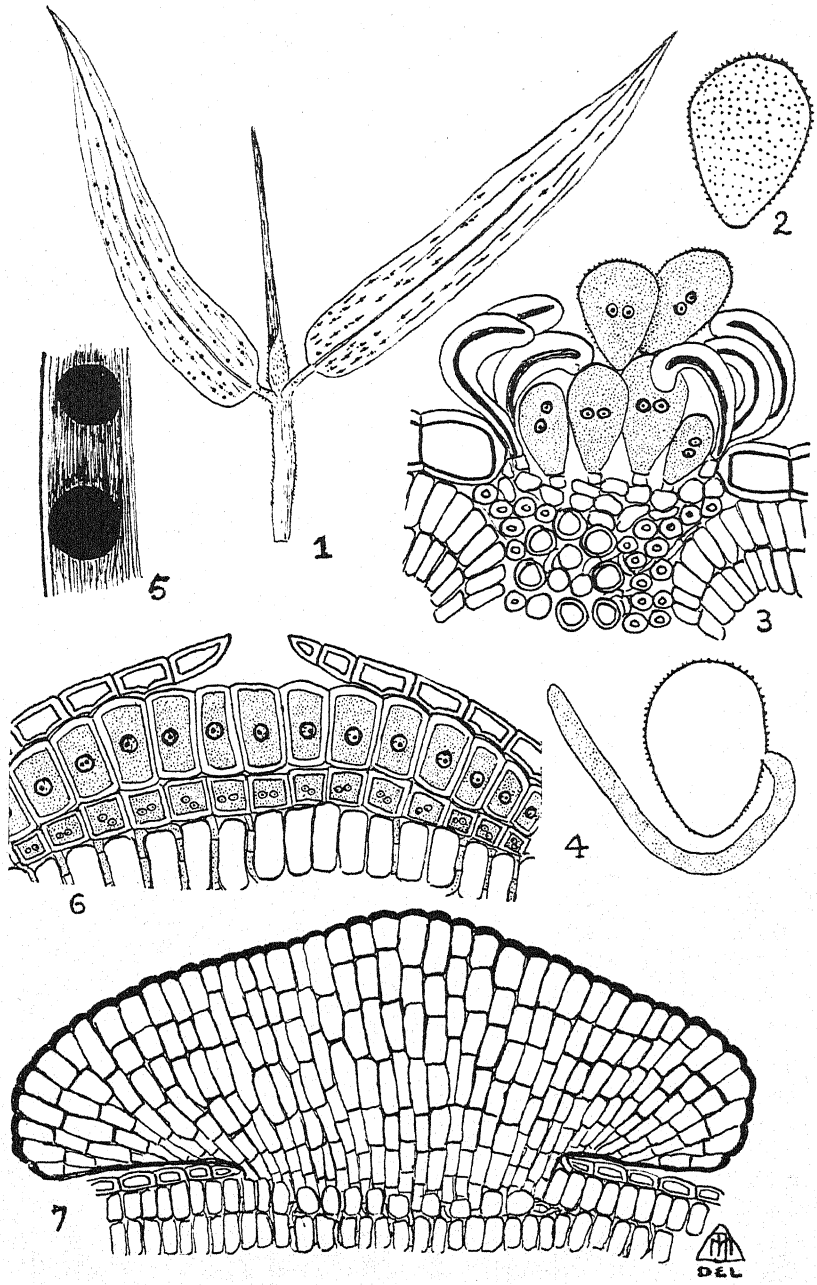
In the course of studies on the rust flora of South India, the authors encountered large tracts of forests in Mysore with the giant bamboo being heavily parasitized by a leaf rust. The rust was found to be *Dasturella divina*, and the identification was checked by comparing it with the type from Herbarium Cryptogamae Indiae Orientalis, New Delhi. Parts of the districts of Kadur and Shimogga in Mysore have large areas covered with the giant bamboo, and a brief survey of these areas indicated the presence of the rust over a wide range. In the present investigation the writers have been successful in establishing the heteroecious nature of the rust by a series of field studies and inoculation experiments. The morphology of the spore forms has been studied with a view to adding more information on the diagnostic characters of the genus and species.

LIFE-HISTORY AND HOSTS.—*Dasturella divina* and *D. bambusina* Mundkur & Khes. were described as hemi forms, the pycnial and aecial stages being unknown. *D. divina* which occurs in Mysore is foliicolous. The uredial stages of the rust develop in abundance on the leaves of the giant bamboo soon after the commencement of the southwest monsoons. The infection spots are evident as minute linear specks on the lower sides of the leaves. The sori are erumpent, striate, and coalesce with one another (fig. 1).

The uredia appear macroscopically as rusty-brown striate sori throwing up large masses of yellowish-brown spores. The rust increases in quantity, as the season advances, through the production of secondary uredia. About the beginning of October, or sometimes as late as November, the telia are first noticed. They are developed at first within old uredia but later are formed independently. The telia are macroscopically black, arising singly or in groups, and appear as flattened pinheads in surface view (fig. 5).

Since pycnial and aecial stages were so far unknown for the genus *Dasturella*, an attempt was made, in surveying the large areas of bamboo forest affected by *D. divina*, to find these stages. Since they are not present on bamboo, attention was focused on a number of unconnected aecia occurring on various hosts in the jungle. The possible oversummering of the rust in the uredial stage was not studied in detail. Since the bamboo shoots remain defoliated for long periods, at which time no viable urediospore inoculum could be collected, chances of the rust perennating in the uredial stage appeared to be slight.

During a number of excursions made by the senior author in the bamboo forests of Mysore, there was noticed a profusion of certain plants bearing aecial stages in consonance with the abundance of giant bamboo bearing uredial and telial stages. In a survey of the locality between Sacrabyle and Megaravalli in Shimogga district, Mysore, he observed the occurrence of an aecial stage in great abundance on *Randia dumetorum* Lam., a member of the Rubiaceae. It was always associated with the giant bamboo rust in the same locality. Only pycnial and aecial stages were present on *Randia*. The infections produced considerable malformations. Pycnial and aecial stages on this host appeared in May and June and always preceded the appearance of the rust on giant bamboo following southwest monsoons. Plants of *R. dumetorum* which



FIGS. 1–7.—Fig. 1, infected leaves of *Dendrocalamus strictus*. Natural size. Fig. 2, urediospore. ×1000. Fig. 3, section through uredium. ×500. Fig. 4, germinating urediospore. ×1000. Fig. 5, surface view of telium. ×80. Fig. 6, section through young telium. ×500. Fig. 7, mature telium. ×250.

grew in places where the giant bamboo did not show rust were all free from this type of infection. This continued association of the rust on *Randia* with the giant bamboo rust led the senior author to suspect that they might be causally connected. Inoculation experiments confirmed this surmise and have established the fact that *D. divina* is a heteroecious eu-form with uredia and telia on the giant bamboo and pycnia and aecia on *R. dumetorum*.

Material and methods

Material for morphological studies of the spore forms were fixed in formalin–acetic-alcohol and in Karpechenko's modification of Navashin's fluid. Microtome sections 8–10 μ thick were stained by Newton's gentian-violet method, with light green as counterstain. The aeciospores, urediospores, and a few of the teliospores were germinated and stained by the method suggested by the senior author (10). Inoculation experiments with urediospores and aeciospores were carried out following the usual procedures. Pieces of leaves bearing mature and freshly erupted uredia and aecia were rubbed on leaves of the plant to be inoculated after first moistening the latter with a spray of water. The inoculated plants were inclosed in moist chambers for 48 hours, after which they were transferred to the greenhouse for observation. The procedure suggested by CLINTON and MCCORMICK (3) for rust infection experiments in Petri dishes was carried out in a number of cases, especially in conducting aeciospore inoculations of bamboo leaves. This method reduces the possibility of contamination from airborne spores, but its success usually depends upon the length of time over which excised leaves are able to retain their fresh state in moist chambers. The bamboo leaves remained fresh for nearly $1\frac{1}{2}$ months—long enough for successful inoculations to be confirmed.

Observations

LIFE-CYCLE ON GIANT BAMBOO

UREDIA.—Some of the morphological details of the spore forms of uredia and telia were given by MUNDKUR and KHESWALLA (6). Uredia are developed in June–July. The sori are hypophyllous, minute, and striate. Sections through the uredium indicate that the sorus is subepidermal (fig. 3), becoming erumpent by the rupture of the epidermis. The sorus is lined along the margin by a fringe of cylindric, deeply incurved paraphyses which inarch over it. The paraphyses are hyaline to pale yellow, rounded at the apex, and possess a central core. The urediospores are sessile, cinnamon yellow, rounded at the apex, obovate to ellipsoid, and measure 17–25×15–24 μ. The germ pores are distinct, and the epispore is covered with dense echinulations (fig. 2).

The urediospores germinate readily when placed in moist chambers, and germ pores become clearly visible as the germ tubes emerge (fig. 4). The two nuclei and the cytoplasmic contents of the spore migrate into the germ tube. The urediospores readily infect the healthy leaves of giant bamboo, causing secondary infections, and are mainly responsible for the spread of the disease.

TELIA.—Telia follow uredia in development in October, appearing as tiny black specks, and can be differentiated with the aid of a hand lens from infection spots of *Phyllachora* sp. which sometimes occur on the same leaves. They arise either singly or in groups. Stages in the development of telia are as follows: From the mass of hyphae com-

posing the basal hymenium beneath the epidermis, a single row of one-celled teliospores is first differentiated. They become laterally coalescent to form a *Melampsora*-like crust (fig. 6). More spores begin to be differentiated from the base, resulting in the production of chains of teliospores. The spores so produced do not become separated from one another but remain fused both laterally and at either end to form a flabelliform crustose head (fig. 7). The epidermis is ruptured and the telial column becomes exposed. No paraphyses are associated with the telia that are developed independently, but in those cases in which telia are formed within old uredia one can observe the marginal fringe of paraphyses. A thin hyphal layer envelops the entire telial head.

Cytological studies indicate that young teliospores are binucleate but that in mature spores the two nuclei fuse (fig. 6). The mature spores are rectangular to cuboid, yellowish brown, smooth, and measure 12.5–30×10–16 μ, with a mean of 20×12.5 μ. The topmost spores of each chain possess apical thickenings which fuse with one another to impart additional firmness to the telial head. MUNDKUR and KHESWALLA studied the telium from herbarium material and considered that it was composed of fasciculate five- to seven-celled teliospores which had coalesced laterally to form a flabelliform crust. They were led to this conclusion by noticing the plane of cleavage of the spores in the crushed telium. It might, however, be pointed out that if the teliospores are considered to be five- or seven-celled, comparable with the condition present in such genera as *Phragmidium*, etc., then all the cells of a teliospore must have arisen through septations of a single teliospore initial and must all be of the same maturity. The developmental stages of the telium observed in *D. divina* in the present study have indicated that the teliospores are produced in catenations, the oldest spore being situated at the outer end and the youngest one at the base. A similar structure for the telium of *D. grewiae* (Har. & Pat.) Thirumalachar has been indicated by one of us recently (11). The telium should therefore be considered as being composed of one-celled spores. The teliospore measurements given by MUNDKUR and KHESWALLA (especially the length of the spore) refer to the entire teliospore chain and not to individual spores.

Only a few cases of teliospore germination have been observed. The spores were found to require a period of rest (3 months), and in germination behavior they are very capricious. The promycelium is external and four-celled, bearing globular sporidia on short sterigmata. Because of the scarcity of germinating teliospores, sporidial inoculations could not be carried out.

LIFE-CYCLE ON RANDIA DUMETORUM

That the aecial stage on *R. dumetorum* might be causally connected with the rust on giant bamboo was first surmised from field studies and was later confirmed by aeciospore inoculations. Sixty-two excised young leaves of bamboo in thirty-one Petri dishes were inoculated with aeciospores from *Randia* by methods already described. The giant bamboo leaves were collected from healthy culms in localities where there were no traces of the rust, and an equal number of leaves was set apart as controls. The inoculations were made on May 10, 1946, when, because of heavy rains, the atmospheric humidity was as high as 90%. The temperature in the laboratory ranged between 24° C. and 30° C.

The first sign of successful infection appeared on the 17th day after inoculation as minute discolored spots. These gradually changed into olive-yellow striate spots by the 22d day. About the 27th or 28th day the sori had developed, throwing up masses of urediospores. In some cases the time required for the development of urediospores was as much as 30–34 days. Urediospores thus obtained were used in inoculating other healthy leaves of giant bamboo, and a second crop of uredia was produced. The spores were also compared microscopically as to structure and measurements with the collections of *D. divina* occurring naturally on the host and were found to be identical. In none of the controls was any trace of infection discernible, and they remained healthy.

The penetration of the aeciospore germ tubes into the giant bamboo leaves was studied in sections. No detailed studies on the pathological histology were made. Sections of bamboo leaves fixed 3 days after inoculation with aeciospores from *Randia* revealed the penetration through the stomata. The germ tube of the aeciospore forms an appressorium on the stoma (fig. 11) and, after penetration, forms a substomal vesicle. Some of the sections of material fixed 15 days after inoculation showed extensive development of intercellular hyphae and the organization of the uredial initials.

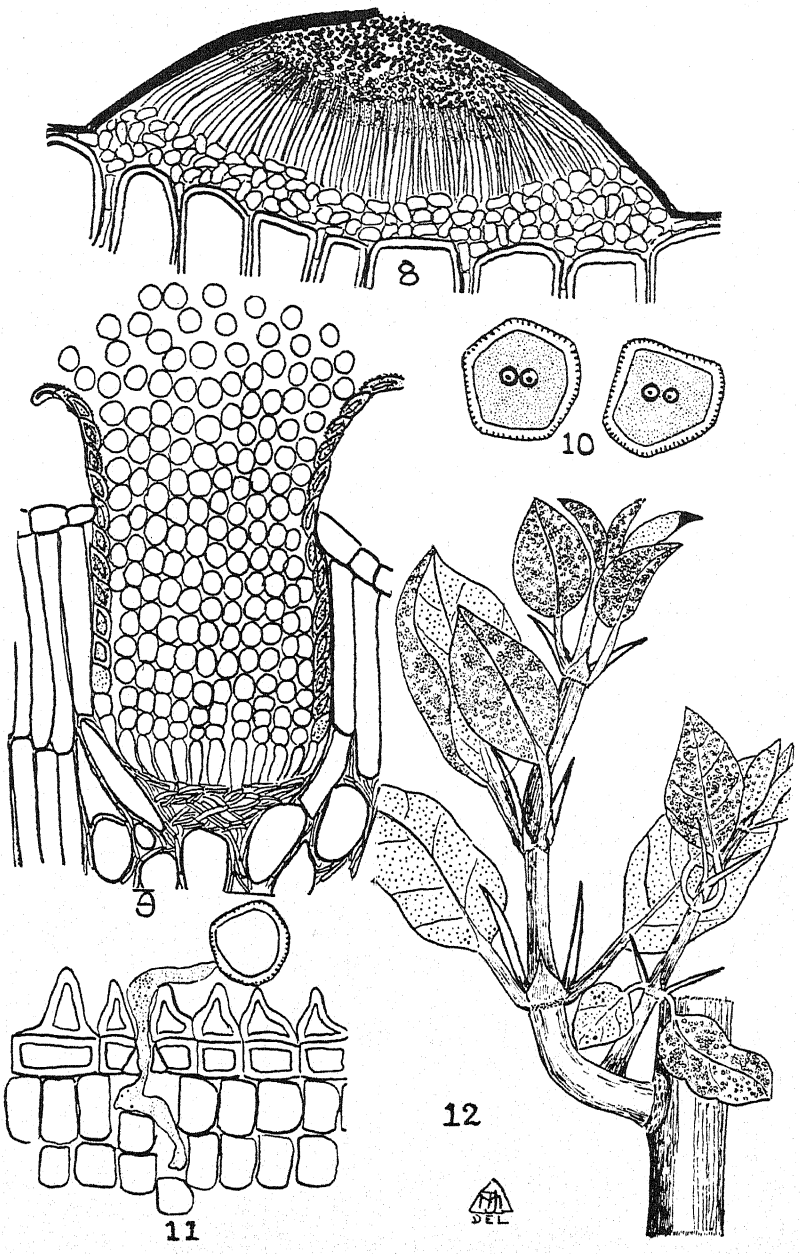
The rust appears on *R. dumetorum* under natural conditions in May and June. At first few leaves are rusted, the infection patches appearing yellow and hypertrophied. Very soon, however, owing to the infection of the meristem in the leaf axils, large witches' brooms showing systemic infection are produced. These witches' brooms have short internodes and a rosette appearance (fig. 12). The bright orange color of the aecia makes the witches' brooms conspicuous in the field.

Pycnia and aecia are developed in abundance on the infected parts. The pycnia are epiphyllous (in the case of leaves, while on the stem they are distributed all over), subcuticular (fig. 8), and appear on the infection patches as tiny orange-yellow specks. They are formed by a uninucleate mycelium and do not possess conspicuous ostiolar paraphyses. The pycniospores are produced in abundance imbedded in nectar.

The aecia are cupulate, hypophyllous (on the leaves), orange-yellow, and possess well-developed peridia (fig. 9). The process of diploidization has not been followed. The binucleate basal cells abstrict numerous spores in succession. Sterile intercalary cells have not so far been observed in the spore chains. The mature aeciospores are subglobose to polygonal, orange-yellow, and show two distinct nuclei in stained preparations (fig. 10). The germ pores are indistinct, and the epispore presents a verruculose appearance because of deep pitting. The spores measure 18–24×15–19 μ. The peridial cells are angular to fusoid, thick-walled, abutting on the sides, smooth on the outer surface and densely rugose on the inner side, and measure 27–40× 15–22 μ.

Discussion

Regarding the identity of the *Aecidium* occurring on the host genus *Randia*, mention may be made of *A. randiae* P. Henn. (5) and of *A. abscendens* Arth. and *A. pulverulentum* Arth. (1). *A. randiae* was described from material collected by Ule in Brazil. The host was cited as *Randia* but was later found to be *Basacantha* (8). *A. abscendens* and *A. pulverulentum* were recorded from Puerto Rico and Mexico, respectively.



FIGS. 8–12.—Fig. 8, pycnium developed on leaves of *Randia dumetorum*. ×800. Fig. 9, aecium. ×250. Fig. 10, mature aeciospores. ×750. Fig. 11, section through artificially inoculated leaf of bamboo with germinating aeciospore. ×500. Fig. 12, portion of witches' broom on *Randia dumetorum* showing systemic infection. Natural size.

The former is parasitic on *R. aculeata* and very closely resembles the pycnial and aecial stages of *Dasturella divina* described in the present study. The pycnia in *A. abscendens* (2) were stated to be subcuticular, the aecia being cupulate and peridiate. The measurements of its aeciospores and peridial cells differ slightly from those of *D. divina*. An *Aecidium* on *R. dumetorum* collected near Sirsi, India, was placed under *A. randiae* by UPPAL *et al.* (12). Since the material was not available for comparison, its identity is not certain.

Pycnial and aecial stages having been found in the present study, the genus *Dasturella* might be described as follows:

Dasturella MUNDKUR & KHESWALLA

Pycnia subcuticular, conoid, without conspicuous ostiolar paraphyses. Aecia subepidermal, cupulate, with well-developed peridia. Uredia subepidermal, with marginal incurved paraphyses and sessile urediospores. Telia subepidermal, erumpent, reddish-brown, in flabelliform heads; teliospores one-celled, developed in basipetal catenations, with the spores united both vertically and laterally to form compact fasciculate crusts; spores germinating after a period of rest by a four-celled external promycelium. Type species: *Dasturella divina* (Syd.) Mundkur & Khes.

The genus *Kweilingia* Teng has been described (9) as parasitic on *Bambusa* sp. The telia of this rust were stated to be erumpent, confluent, and crustose, with teliospores occurring in chains of one-celled spores which were laterally united. The rust was first described by TENG as *Chrysomyxa* and later on was accommodated in his new genus *Kweilingia*. This rust is a microcyclic form and is distinctly separate from *Dasturella*.

The genus *Dasturella* was placed by MUNDKUR and KHESWALLA under the tribe Raveneliae of DIETEL (4) on account of its fasciculate sessile telial heads, resembling to some extent those of *Nothoravenelia* Diet., a member of that tribe. In general, however, the members of that tribe possess multicellular telial heads which are not only erumpent but become pulverulent. The presence of cysts in *Nothoravenelia*, characteristic of most of the Raveneliae, would seem to remove the genus *Dasturella* (which lacks them) farther from that tribe. It appears more reasonable to accommodate it in the tribe Ochrosporae of DIETEL's classification of the Pucciniaceae along with *Cerotelium*. The characters enumerated for the Ochrosporae are the presence of subcuticular pycnia, peridiate cupulate aecia, uredia with hyphoid paraphyses or peridium, and telia in crusts, often with catenate spores. All these characters are well represented in *Dasturella*. The telial crusts of *Cerotelium*, although columnar at the base, become pulverulent at the apex, with the spores getting separated by germinating. In *Dasturella*, on the other hand, the teliospores are firmly united to retain their flabelliform shape and are resting spores.

Summary

1. *Dasturella divina* (Syd.) Mundkur & Kheswalla is a heteroecious eu-form with uredia and telia on the giant bamboo (*Dendrocalamus strictus* Nees), and pycnia and aecia on *Randia dumetorum* Lam. (Rubiaceae). The causal connection between the stages of the life-cycle on *Randia* and on the giant bamboo was first surmised from the continuous association of the two rusts in bamboo forests and was later confirmed by inoculation experiments.

2. Infections on *Randia* produce marked hypertrophy and formation of witches' brooms following systemic invasion of the host. The pycnia are subcuticular and conoid, borne on haploid mycelia. The aecia are cupulate and

peridiate, and the aeciospore chains lack sterile intercalary cells.

3. Uredia and telia occur on the leaves of giant bamboo. The sori are striate, and morphological details of these two spore forms have been given by MUNDKUR and KHESWALLA. The telia, which occur in flabelliform erumpent crusts, are, however, contrary to their description, composed of one-celled, catenate spores produced in basipetal succession. The accommodation of the genus in the tribe Ochrosporae of DIETEL is suggested. Aeciospores from *Randia* were found to infect the leaves of the giant bamboo, inciting the rust on the bamboo. The period of incubation ranged from 27 to 34 days. The urediospores spread the disease by secondary infection.

MALLESWARAM, BANGALORE, INDIA

LITERATURE CITED

1. ARTHUR, J. C. New species of Uredinales V. Bull. Torrey Bot. Club 33:513–522. 1906.
2. ———. Uredinales of Porto Rico based on collections by F. L. STEVENS. Mycologia 7:315–332. 1915.
3. CLINTON, G. P., and MCCORMICK, F. A. Rust infection of leaves in Petri dishes. Connecticut Agric. Exp. Sta. Bull. 260. 1924.
4. DIETEL, P. Hemibasidii. *In* ENGLER and PRANTL's Die natürlichen Pflanzenfamilien (Aufl. 2) 6:24–98. 1928.
5. HENNINGS, P. Beiträge zur Pilzflora Südamerikas I. Hedwigia. Pp. 202–262. 1896.
6. MUNDKUR, B. B., and KHESWALLA, K. F. *Dasturella*, a new genus of Uredinales. Mycologia 35:201–206. 1943.
7. SYDOW, H. Über zwei neue Arten der Gattung *Angiospora*. Ann. Mycol. 34:69–73. 1936.
8. SYDOW, H., and SYDOW, P. Monographia Uredinearum IV. Leipzig. 1924.
9. TENG, S.-C. Supplement to higher fungi of China. Sinensia 11:105–130. 1940.
10. THIRUMALACHAR, M. J. A method for germinating and staining teleutospores. Jour. Indian Bot. Soc. 19:71–75. 1940.
11. ———. Notes on three South African rusts. Bull. Torrey Bot. Club 73:346–350. 1946.
12. UPPAL, B. N.; PATEL, M. K.; and K MAT, M. N. The fungi of Bombay. Bombay (India). 1935.

GROWTH AND MORPHOLOGY OF TOBACCO TISSUE CULTURES IN VITRO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 580

SAMUEL M. CAPLIN

Introduction

A milestone in botanical research was reached in 1939, when WHITE (22), using stem tissue from tobacco, and GAUTHERET (4) and NOBECOURT (13), using cambial tissue from carrot root, showed independently that excised plant tissues will grow indefinitely if they are repeatedly subcultured to fresh nutrient of the proper constitution. After HABERLANDT (8) had formulated the idea of a plant tissue culture at the beginning of the century, the first big step was made when ROBBINS (15, 16, 17) and KOTTE (11, 12) attempted independently to grow excised root tips and stem tips in nutrient solutions of various composition. By repeated subculture ROBBINS (16) obtained a decreasing growth rate for root tips which suggested that unknown material not present in the nutrient might be essential for continued growth. Although GAUTHERET (7) learned much about nutrient requirements by attempting to grow cambium from large trees, WHITE (18, 20, 21)

measured growth of excised root tips and used new information from nutrition and biochemistry to develop a synthetic nutrient for the continued growth of excised roots. This done, he was able to culture excised stem tissues (22). On the surface of an agar medium the latter grew in an amorphous, undifferentiated state, and by periodic subculture to fresh nutrient they were shown to possess an unlimited capacity for growth. Detailed historical reviews have been adequately given by WHITE (19, 23, 24).

Further investigation of the conditions necessary for growth of plant tissue cultures was undertaken by HILDEBRANDT *et al.* (9, 10). In order to compare various treatments, they cut cultures into pieces approximately 20–30 mg. in weight, allowed them to grow for 6-week periods, and determined the final fresh weights. Through the use of sufficient tissues and replications and statistical analysis of the data, they were able to work out the optimal temperatures, pH, and sucrose concentrations (9) and the optimal concentrations of the other constituents of a nutrient for tobacco and of one for sunflower callus cultures (10). Their methods and results were necessary groundwork to further research involving the culture of excised plant tissues.

Thus far work by various investigators has resulted in (*a*) obtaining plant tissues which will grow in culture and (*b*) determining the optimal conditions for their growth. Now that these problems have been partially solved, more attention can be directed to investigation of the nature of the growth process itself in cultures of excised plant tissue. The experiment described here concerns the mechanism of increase in size of a tobacco callus culture and its rate of growth.

Material and methods

The tissues used were 6-week-old subcultures generously supplied by Dr. A. C. HILDEBRANDT from a stock culture which he obtained in 1941 from Dr. P. R. WHITE. WHITE has described (18) the isolation of the original cultures from fragments excised from a region about 5 cm. from the stem tip of *Nicotiana glauca* Grah. ♀ × *N. langsdorffii* Weinm. ♂. Of the original explants, only those which actually contained cambial tissue continued to grow.

The nutrient used was the modification by HILDEBRANDT *et al.* (10) of WHITE's semisolid medium for tobacco callus tissue, the composition of which was kindly imparted by Dr. HILDEBRANDT prior to publication of their report. Salts and sucrose were reagent grade, and redistilled water was used in preparing the nutrient. Difco brand "Bacto Agar" was used at 0.5% concentration. Experimental cultures were grown in the dark in 125-ml. Erlenmeyer flasks containing 50 ml. of medium. For the first 3 weeks they were in an incubator at 26° ± 1.0° C. and were then transferred, because of approaching summer, to a thermostatically controlled refrigeration unit equipped with a heater operating at this temperature.

Tissues were cut into uniform cubes by means of a dicer consisting of a set of razor blades separated by flat washers carefully measured with a micrometer caliper and held together with small bolts. Two such instruments were made; one had a distance between blades of 1.7 mm., and the other of 2.7 mm. For practice a block of potato was cut into slices, the slices into shoestrings, and the shoestrings into cubes. The weights to the nearest 0.1 mg. of the first thirty cubes of potato picked at random from

each group are shown in table 1. The lesser variability of the larger cubes can be ascribed to variation in the distance between blades during the cutting process; these variations were relatively greater in the smaller cubes.

Experimental

Fifteen tobacco cultures 10–15 mm. in diameter were cut into cubes with dicer A, and ten cultures with dicer B; within each size group all cubes were mixed. Each culture flask was then inoculated with four cubes and was placed in the culture chamber. After contaminated cultures had been eliminated, 168 cubes remained in group A, 71 in group B. At weekly intervals four or five culture flasks in each group were removed; the cultures were rinsed in water, drained on moist filter paper in a covered Petri dish in order to prevent drying, weighed individually, and preserved in formalin–acetic acid–alcohol. Later, in order to determine the sequence of events during the early stages in growth of a cube, additional cultures were cut with dicer A and placed under the same experimental conditions. These cultures were removed at 2-day intervals over a 12-day period and were preserved in Navashin's solution.

Results

Morphology and histology

Growth of the cultures from small cubes during the course of 9 weeks is illustrated in figures 1–4 and is represented diagrammatically in figure 16, which shows how the cultures changed from cubes into somewhat flattened hemispheres.

When a tissue cube was placed on the nutrient medium, the first divisions of cells occurred by the fourth day (fig. 9). All stages of mitosis were found in the upper portion of the cube to a depth of about seven cells. Further divisions resulted in the proliferation of rows of cells (figs. 5, 10), which became organized into knob-like protuberances (figs. 3, 5, 6).

By the end of the second week the cubes had visibly changed in appearance, the exposed surfaces being covered with small knobs, or bumps, about 1 mm. or less in diameter. As the cultures grew larger, these knobs increased in number.

TABLE 1

Dicer	Distance between blades (mm.)	Range (mg.)	Mean (mg.)	Standard deviation	Coefficient of variability (%)
A...	1.7	4.0– 6.0	5.23	0.521	10.0
B...	2.7	18.0–22.0	19.60	0.732	3.7

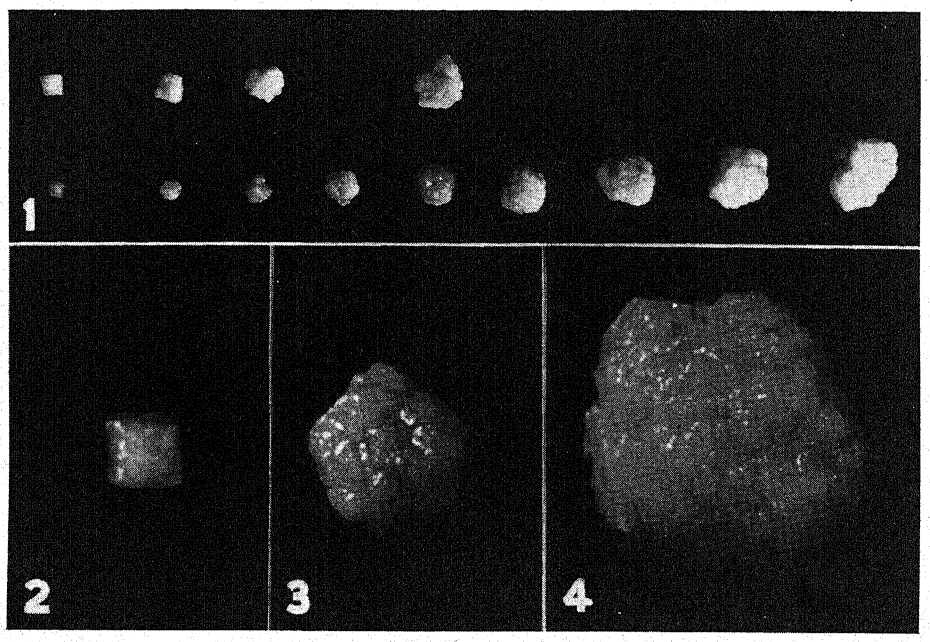
On a 4-week-old culture they were rounded and more or less regularly spaced (fig. 3); later the knobs were crowded together as if in competition for the surface available (fig. 4). As the cultures grew still older, the knobs often became obscured by the presence of dead cells, which gave to the surface a white, granular appearance.

Sections through cultures from successive weeks in the growing period indicated that the knobs increased in number in two ways. One method was by a process of cleavage (fig. 7), which appeared to be the result of failure by a cell, or a row of cells, at the surface to continue dividing. Instead the cell (or cells) enlarged greatly, while other cells at the sides continued to proliferate. The other method was by the formation of new knobs in the subsurface of existing ones (figs. 11, 13).

Growth of a knob was accomplished by divisions of cells at and near the sur-

face (figs. 6, 12). In these divisions new walls were laid down tangentially to the surface. The newly formed cells toward the center of the tissue mass increased in size and, in so doing, pushed the actively dividing cells out along a radius. In this manner a knob continued to grow, its cells forming in orderly rows (figs. 5, 6). Not all divisions within the knob, however, occurred tangentially. As examination of figures 6 and 12 shows, the knobs increased not only in length but in diameter as well. This was effected by cell divisions which occasionally occurred radially (figs. 6, 10, 12) and generally in the center of the knob. New rows of cells were thus formed, which account for the fan-shaped appearance of the knobs in radial section. As the knobs grew, they became appressed laterally, and some of the surface cells in the area of contact were crushed flat (figs. 6, 12). Such crushed cell material stained deep red with safranin. Other cells from adjacent knobs formed such intimate contact that, when a culture was snapped in two mechanically, very often the split occurred through knobs rather than around them.

That proliferation from a cube occurred only from the upper portions suggests that some factor is important in determining where regeneration will occur. A meniscus of liquid nutrient reaching to the top edge formed around the cube soon after it was placed on the agar medium. Thus, a low supply of oxygen may have been effective in limiting growth in the lower portions of the culture. Whether polarity plays a role in this response, however, either partially or even entirely, must await future investigation.



FIGS. 1–4.—Largest cultures. Fig. 1, each weekly series; left to right, 0, 2, 3, 4, 5, 6, 7, 8, and 9 weeks. ×1. Fig. 2, freshly cut cube. Fig. 3, fourth week. Knobs on culture are evenly spaced. Fig. 4, eighth week. Knobs appear crowded. Figs. 2, 3, and 4, ×5.

Examination of cross sections of cul-

tures indicated, despite equal amounts of growth, that not all the sub-surface meristems were in an active state of division at the same time (fig. 6). This raises the question as to whether the knobs grew continuously or in an intermittent and possibly rhythmic fashion. No information is available on this point.

Differences among cultures of same age

The growing cubes, though uniform in initial size, varied considerably in weight at each sampling (table 1). Attempts to detect a relationship between histological aspects and size to account for these variations in amount of growth were not very successful. Certain differences among cultures were noted, however, which might provide clues for an explanation of differences in size and which may form a possible basis for further work.

Differences among cultures at any one time were found in their staining reactions. In some cultures the cell walls in the area approximating the original cube (i.e., in the portion remaining which did not proliferate) were stained deeply red with safranin, although the

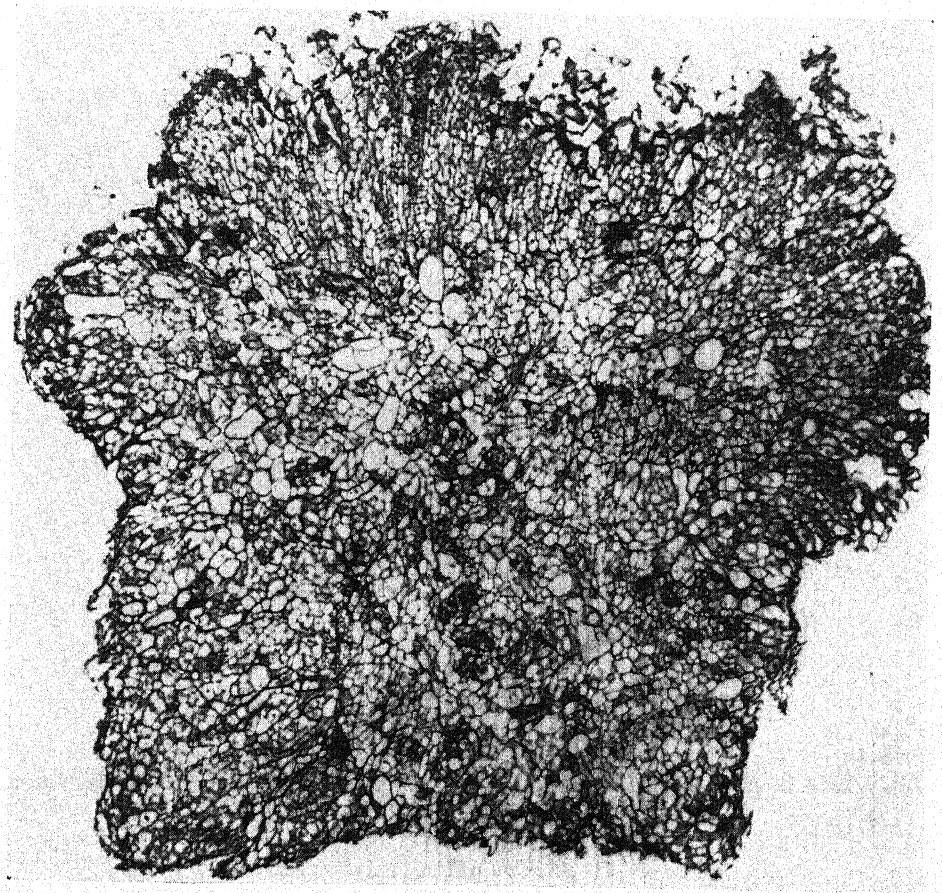


Fig. 5.—Section through 3-week-old culture cut perpendicular to nutrient agar surface, showing that proliferations on culture consist of cells which have been laid down in rows.

walls of the newly proliferated cells were not. This condition was found in large as well as in small cultures of the same age.

Sections through some of the larger tissue masses in the older age groups showed scattered, apparently dormant, meristematic areas or patches (fig. 14), although the exact way in which they were formed is not clear. Possibly they were cell groups which failed to enlarge after their formation, retaining a meristematic appearance. However, a number of the larger patches seemed to be somewhat active. Mitotic figures found occasionally within these areas indicated that new cells were being formed, and the crushing of a number of cells around these areas indicated that enlargement of some of them was also occurring (fig. 14). Little increase in the weight of a culture, however, can be attributed to the formation of these groups of meristematic cells; if these regions had been

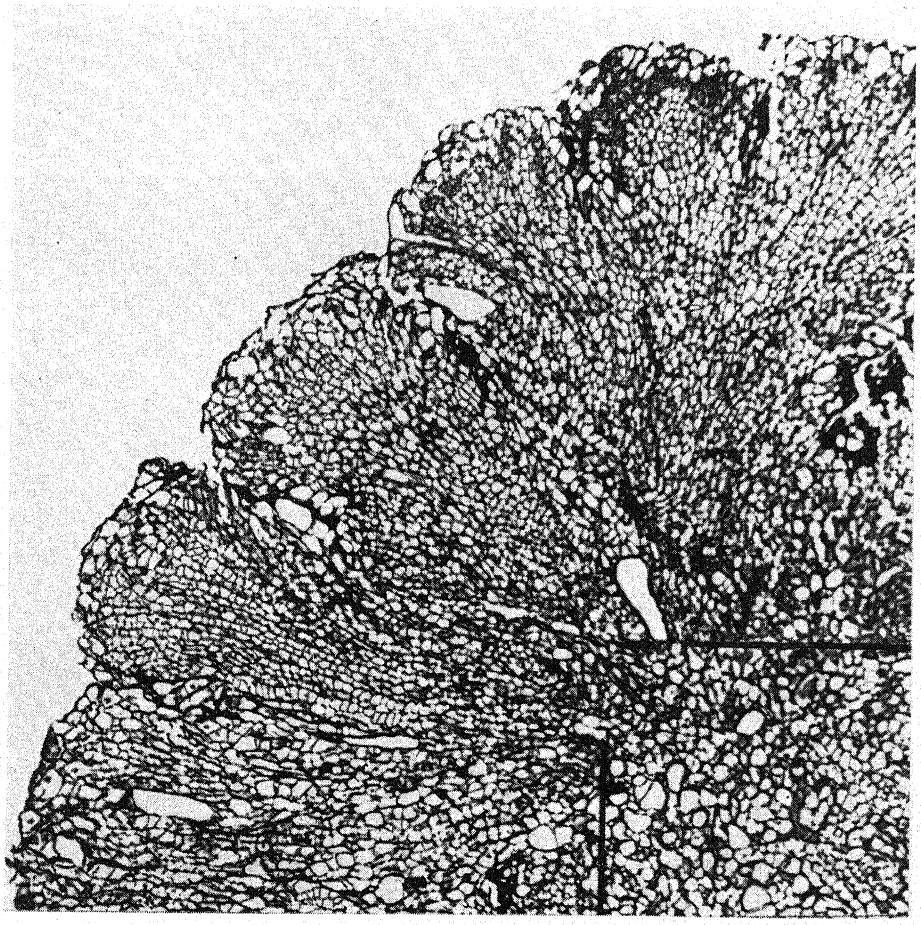
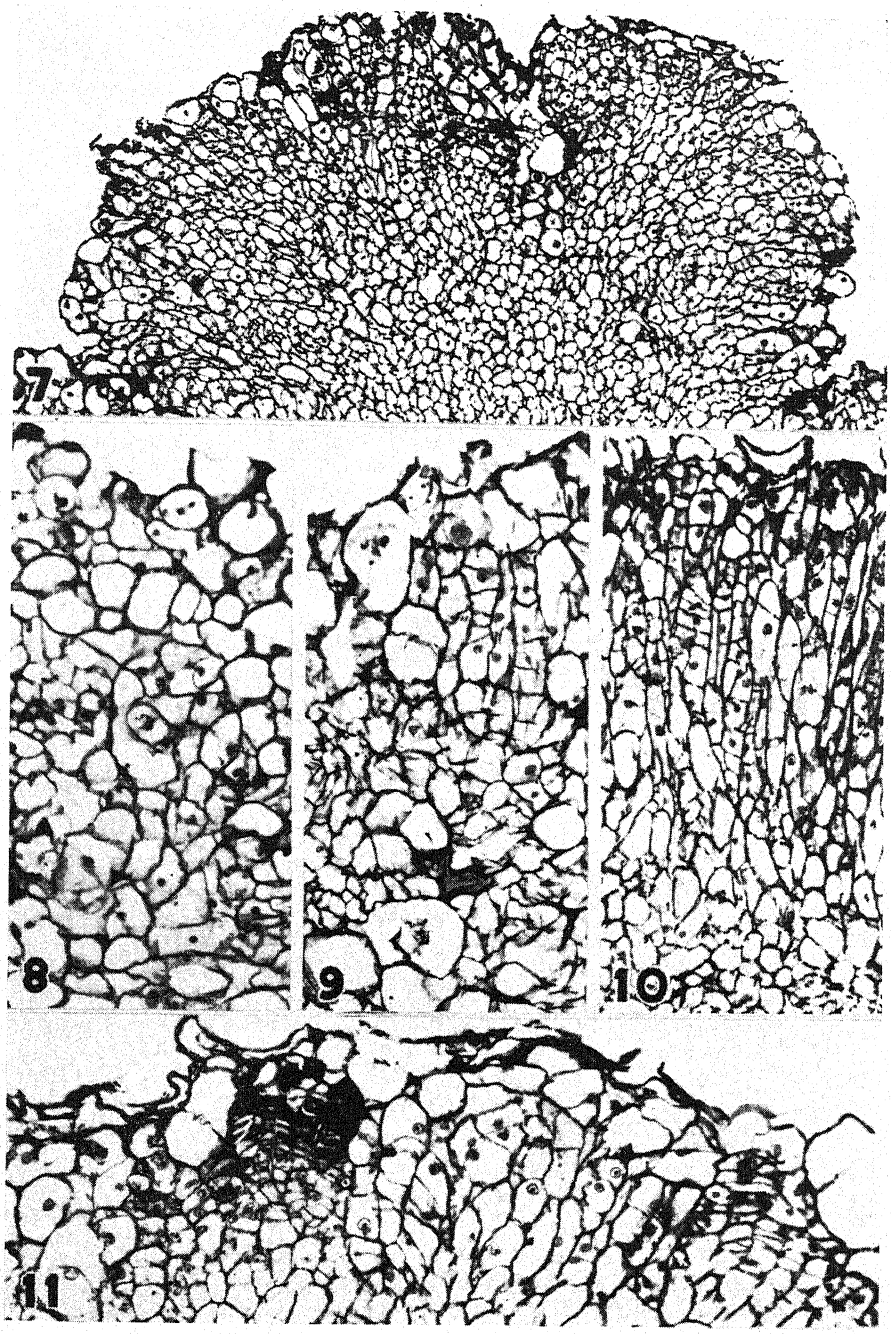
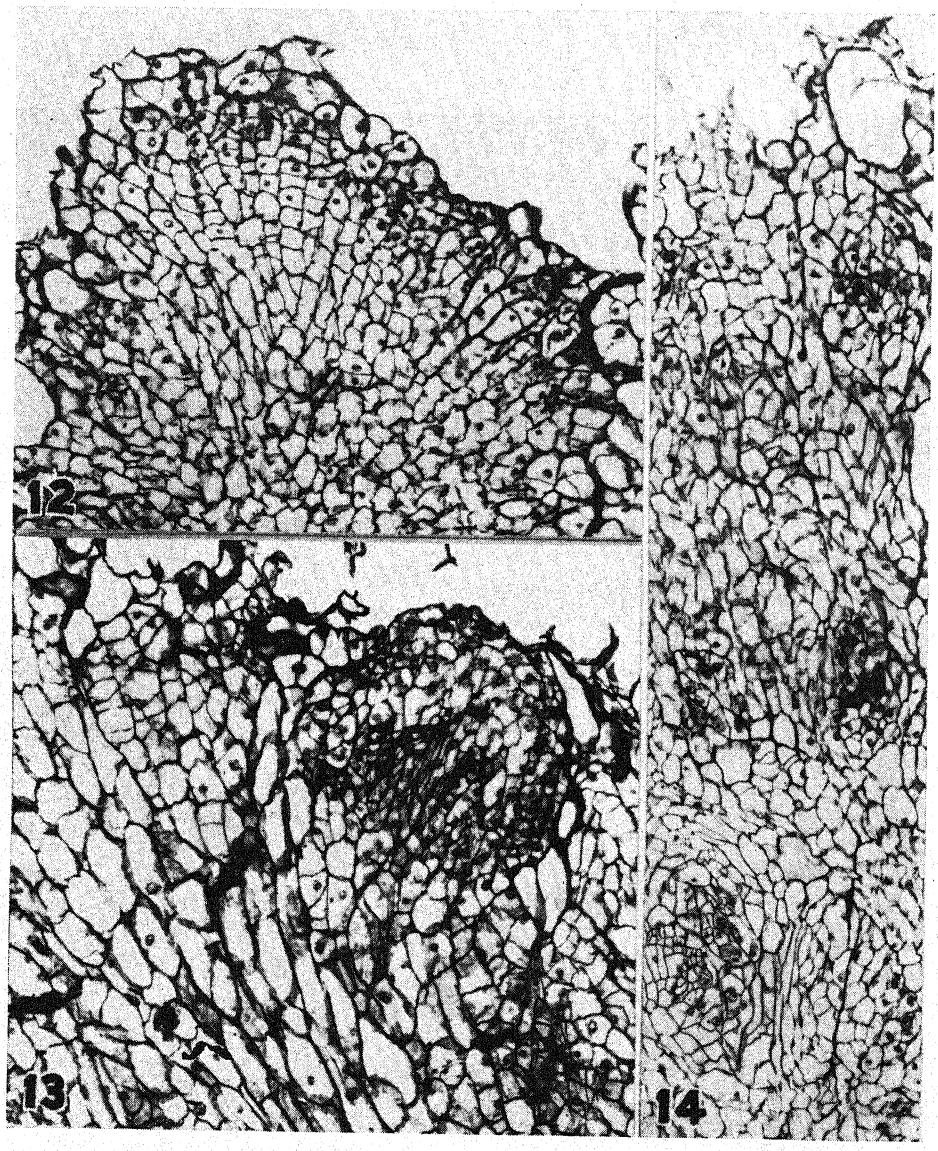


FIG. 6.—Section through 6-week-old culture cut parallel to nutrient agar surface (one-fourth of section shown; lines drawn indicate portion remaining of cube from which culture grew). Orderly growth has resulted from meristematic activity at surface and subsurface of culture. Knob at upper right is in active state of division (see also fig. 12).



FIGS. 7–11.—Fig. 7, knob undergoing cleavage to form two knobs. This process appears to be initiated by failure of one or more of dividing cells in subsurface of growing knob to continue dividing. Instead it enlarges greatly, while neighboring cells continue to divide and grow around it. Figs. 8–10, sections through cultures of different age. Fig. 8, freshly cut cube. Fig. 9, 4 days old; cell divisions tangential to surface have resulted in formation of cells in rows. Fig. 10, 12 days old; continued cell division has produced outgrowth; radial divisions have formed new rows of cells. Fig. 11, new meristematic knob forming in subsurface of older knob.



FIGS. 12–14.—Fig. 12, knob showing cell divisions at and near surface. By continued division and enlargement of cells, structures such as this continue to push out, as shown in figs. 5 and 6. Fig. 13, young knob formed below surface and rupturing tissue of old knob outside it. Fig. 14, section through culture (from ninth week) having a somewhat less orderly histological pattern than culture shown in fig. 6. Organized rows of generally mature parenchyma cells are broken up by patches of meristematic cells formed by continued division of some cells deep in culture. Note crushing of adjacent cells around lower patch.

growing actively enough to contribute appreciably to the increase in weight, breaks and tears, which were not evident, would have been found. Some sections through large, older cultures had a bandlike formation of meristematic cells located along the side of a knob where adjacent knobs were in contact. Of such adjacent knobs the side of only one was found to be meristematic.

The cultures were growing in the dark and were therefore dependent on the medium for carbohydrate. The larger cultures in a given age group were found to contain starch; the smallest ones, however, were found to be free of starch. The areas containing starch were located near the surface in the same general position as the subsurface meristematic cells. In these areas cells with mitotic figures were occasionally found among cells containing large



FIG. 15.—Enlargement of young knob in fig. 13, showing "wound tracheids" in center. From serial sections there was no indication of connection between these groups of cells and other similar groups occurring elsewhere in the culture.

starch grains, which is not the normal condition in the apical growing region of angiosperms. It remains to be seen what is the causal connection, if any, between the ability to deposit starch and the greater growth of the large cultures.

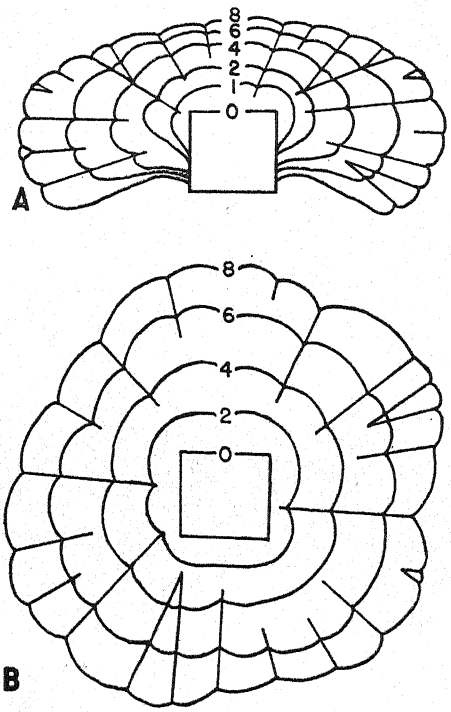


FIG. 16.—Diagrammatic sections through culture illustrating morphological changes during 8 weeks' growth. *A*, section perpendicular to nutrient agar surface; *B*, section parallel to nutrient agar surface. Cubes changed into somewhat flattened hemispheres by growth of knobs in which proliferation of cells occurred in and near surface. Concentric lines represent shape of culture after indicated number of weeks' growth. Radial lines indicate surface of contact between adjacent knobs. Points of radial lines nearest center are points at which knobs have divided. Where two lines radiate from such points, new knobs have been formed.

DIFFERENCES FROM WHITE'S CULTURES

The tobacco cultures first isolated by WHITE grew in a much less organized manner than those here described. A section through an 8-week-old culture illustrated by WHITE (22) shows a relatively uniform central area surrounded by loosely organized groupings of meristematic knobs generally separated from one another by groups or rows of much larger cells. Sections through our cultures show, however, much more evidence of growth in an orderly fashion, whereas growth in WHITE'S original cultures seems to be more randomized. The reasons for these histological differences are not clear. The fact that WHITE'S cultures grew in the light rather than the dark may, however, be a factor.

Another difference between the two sets of cultures concerned the presence of differentiated cells of the wound tracheid type. Whereas in WHITE'S cultures these were present as "isolated" cells, here serial sections indicated that at least for short distances many of these cells were connected together and similarly oriented. Figure 15 indicates that the formation of these cells may occur very early in the development of a newly formed meristematic knob.

WEIGHTS OF CULTURES

The weights of the cultures increased with time, but the increase of the largest culture from each weekly sample, in both the A and the B groups, was much greater than the average increase for each group respectively (table 3). In group A after 9 weeks there had been an 18-fold increase in mean weight, while the largest cultures showed a 34-fold increase; in group B after 5 weeks there had been a 4.8-fold increase in the mean, and a 6.1-fold increase in the largest cultures. This variance also increases with time as the cultures grow. Only three cubes in group A and one cube in group B failed to grow at all.

When the weights of the largest cul-

tures from each weekly group sample were plotted, it was discovered that they fell in regular logarithmic relationship to one another. This indicates not only that growth was proportionately progressive but that the largest culture in each weekly sample of twenty cultures had the *maximum* growth rate in its group; it also had the *same* growth rate as the largest culture from each successive weekly sample. Each point of each growth curve in figure 17 represents, therefore, the growth attained by a single culture of maximum growth rate under the conditions of the experiment. The largest cultures in successive weeks in the A and B series are shown in figure 1. The growth curve of the A cultures shows an anomalous weight at 2 weeks, which at present cannot be accounted for except by assuming that the particular culture up to that time had a growth rate the same as the largest cultures in the other weeks and that its initial weight (which was not measured) was approximately 20% greater than the largest measured initial weight at time of inoculation.

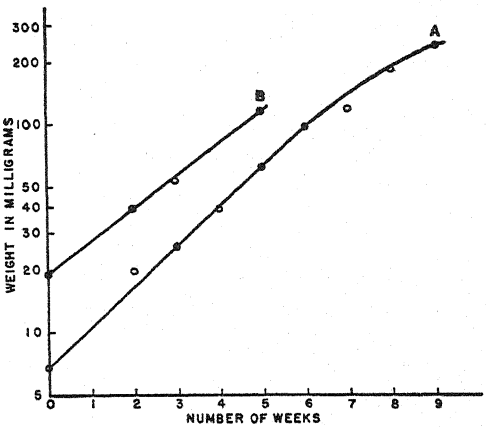


FIG. 17.—Log of wet weight in relation to time of largest cultures in A and B series.

Discussion

TISSUE GROWTH AND COMPOUND-INTEREST LAW

BLACKMAN (1) has interpreted the addition of new material by a plant in terms of the compound-interest law. The equation which BLACKMAN found applicable for the growth of an annual plant is

$$W_1 = W_0 e^{rt},$$

where W_1 is the final weight, W_0 is the initial weight, e is the base of natural logarithms, r is the rate of interest, and t is the time. The rate of interest is expressed as percentage increase per day. It is considered by FISHER (3) not only to be a valuable index for comparing the efficiency of different plants over comparable times and phases of development but as the correct measure for the mean value of the relative growth rate over any period, long or short. For a constant growth rate during a time interval this would be a way of describing numerically the relative rate of growth; for a fluctuating or changing rate of growth during the time interval, such as the alternating growth rate in day and night or a change in average daily growth rate during a growing period, this would represent an average relative rate of growth for the period. BLACKMAN found that rates of interest for a number of plants, calculated from the data of other workers, ranged from 10.6 to 20.5% per day.

The use of BLACKMAN's formula appears to be particularly applicable for describing the growth of the writer's tissue cultures, since under the easily maintained uniform conditions of the experiment the weights of the largest cultures appear for the first 5 or 6 weeks to indicate constant exponential growth.

Applying the formula, which is more

convenient in the form $\log_e (W_1/W_0) = rt$, r may be calculated as shown in table 2.

The relative growth rates of 6.3% and 5.15% per day represent the maximum rates of growth of the cultures of different initial size under the conditions of the experiment. It should be noted here that the tissues were not subcultured weekly but were growing continuously in one place on the nutritive medium.

Thus far little attention has been given to the quantitative interpretation of the growth rates of plant tissue cultures.

TABLE 2

Group	No. of days (t)	Relative increase	r
A.....	42	14.2	0.063
B.....	35	6.1	0.0515

However, some data showing weight increase of tissues in a given time are available. NOBECOURT (14) reported that a 1-mg. fragment of carrot root tissue increased to 185 mg. in 5 months, with a transfer to fresh nutrient after the first 3 months. The rate of increase calculated from BLACKMAN's formula was 3.48% per day. GAUTHERET (5) measured the increase in weight during the eighth monthly passage of eight pieces of carrot root tissue. These varied in size at the beginning of the culture period from 50 to 180 mg. Average rates of increase for 58 days ranged from 2.8 to 4.9% per day. Data on three tissues from the ninth passage were also given. In 18 days the most rapidly growing culture grew from 125 mg. to 445 mg.—an increase of 7.05% per day. Later he reported that a 0.5-mg. fragment of carrot tissue increased to 15 gm. in a period of 10 months—an increase of 30,000 times. This represents an increase of 3.4% per day. Since nothing is known in these cases concerning the growth curves during the culture period, it is assumed that these were the average growth rates and that the maximum rates of growth may have been greater. However, the calculated figures represent the maximum rates of growth if the rates of increase were constant.

In his paper describing the first successful culture of *Nicotiana* callus, WHITE (22) reported an average 3-fold increase per passage of 7 days. No figures were cited. Growth on this basis is a rate of increase of 15% per day, a figure comparable with that for the relative growth of an intact plant. Additional information concerning growth rates in plant tissue cultures must await further investigation.

VARIATION WITHIN GROUPS IN GROWTH RATES OF CULTURES

Table 3 shows that weights of cultures in groups A and B increased progressively in variability as the experiment proceeded. Initially all the cultures within each group were approximately the same size and shape. Had they been growing at the same rate, one would, at the end of a growing period, expect the variability of the cultures—expressed in standard deviation—to increase only in proportion to the increase in weight. The coefficient of variability would therefore have remained constant. This, however, was not the case. As the cultures increased in age, their variability increased considerably in both groups A and B. This effect was so pronounced that by the end of the eighth week the weight ratio of the largest to the smallest culture in group A was 10:1.

This increase in variability may be accounted for by either one of the following hypotheses: (*a*) the cultures were all growing at different rates or (*b*) they were all growing at the same rates but

different cultures stopped growing at different times after the beginning of the experiment.

Work on this phase of the problem is at present in a preliminary stage. Mention may be made, however, of an experiment in which tobacco callus cultures having an initial weight of 9 mg. or less were grown and weighed individually at weekly intervals during the growing period. The data collected seem to indicate that (*a*) is the correct hypothesis. Of fourteen pieces cultured for 11 weeks, none had stopped growing during that time. All cultures had increased progressively in weight. At the end of 11 weeks the ratio of the largest to the smallest culture in the group was as 2088:210 mg., or 10:1. At the beginning of the experiment these cultures had weighed 8.8 mg. and 8.4 mg., respectively. Of these, one culture had increased 238 times, the other only 25 times. It seems, therefore, that small cultures of this tobacco callus possess inherent differences which are independent of initial size.

On the basis of these observations the differences in growth rates of initially small cultures must be due to inherent differences in the tissue cubes at the time that they were cut. To what extent these differences between tissue cubes are associated with their original sites or orientation in the tissue mass when cut, or with the growth rate of the culture from which they were subcultured, or to what extent the age of a culture is a factor in regulating subsequent growth rates, are questions yet to be investigated.

Differences in growth rate in relation to initial size of explant

Examination of figure 17 indicates that the growth curves do not have the same slope. Cultures of larger initial size (B) have a smaller relative growth rate. The difference between the two slopes is 1.15% ($P = < 0.005$ for $N = 6$). Although this percentage may seem small, such a difference can have a profound effect on the final weight. As Blackman (1) pointed out, a difference of 1% in relative rates of growth will cause a 2-fold difference in final weight at the end of 69 days, when the rates of growth have remained constant. This suggests that cultures of still smaller initial weight and possessing the same

TABLE 3

GROWTH OF UNIFORMLY CUT CULTURES OF TOBACCO CALLUS

No. of weeks' growth	No. of cultures removed	Weight of largest culture (mg.)	Mean weight (mg.)	Standard deviation	Coefficient of variability (%)
	A cultures				
0.....	20	6.8	5.8	0.61	10.5
2.....	20	19.9	10.6	3.28	31.0
3.....	20	26.0	17.6	4.74	26.8
4.....	20	39.0	19.8	6.26	31.7
5.....	20	63.8	36.6	16.80	46.0
6.....	20	96.7	46.4	22.30	48.0
7.....	16	119.6	72.0	27.60	37.8
8.....	16	184.0	93.0	37.50	40.3
9.....	16	231.4	105.0	57.20	54.5
		34×*	18×*		5×*
	B cultures				
0.....	20	19.1	17.2	0.75	4.6
2.....	19	39.7	32.7	3.38	9.4
3.....	16	53.8	43.4	6.27	14.5
5.....	16	116.0	74.4	22.30	30.0
		6.1×†	4.8×†		6.5×†

* Increase in 9 weeks.

† Increase in 5 weeks.

physiological potentialities would show an even greater relative growth rate.

GAUTHERET (6) has presented evidence which supports this point of view. Using cultures of carrot root tissue, he found that the ratio of the final to the initial weight of a culture varied inversely as the initial weight. From data in his earlier paper (5) on carrot tissues grown for 58 days, it can be shown by arrangement of his data in order of increasing initial weight—50, 50, 110, 115, 150, 155, 160, and 180 mg., with final weights of 585, 735, 1180, 815, 1265, 1270, and 915 mg., respectively—that the relative rates of growth fall into a generally descending series as follows: 4.95, 4.63, 4.10, 3.36, 3.74, 3.62, 3.57, and 2.81%, respectively.

The concept that cultures of smaller initial weight tend to grow relatively more rapidly is further supported by the work of BUCHSBAUM (2) in studies with embryonic cells of the chick. He showed that smaller explants had greater relative increase in 48 hours than larger explants. He attributed this slower growth in larger explants to the inhibiting effect on peripheral cells of the more unfavorably located interior cells, owing to slow diffusion of nutrients and metabolic products.

That these factors operate in plant tissue cultures as well is doubtless true. However, they do not appear to be the complete answer, for, as figure 17 indicates, cultures of the same weight from each of the curves maintained different growth rates for well over 3 weeks. Yet there is every reason to believe that the cultures of maximum growth rate in each group were initially of the same physiological constitution and differed only in size. This difference in growth rate may perhaps be accounted for by the difference in shapes of cultures of similar weight in the respective groups; at 20 mg., for example, culture B was approaching a hemisphere in shape, while culture A was still a cube.

Summary

1. The growth in darkness of tobacco stem callus tissue on nutrient agar medium was investigated. Growth was studied during a period of 9 weeks in cultures taken from stock subcultures which were 6 weeks old. The experimental cultures were prepared by cutting the stock cultures into uniform cubes of two different sizes by a technique which is described.

2. Increase in size of the cultures resulted from the proliferation of small knoblike protuberances in which division and enlargement of cells was occurring very close to the surface. Knobs increased in number in two ways: (*a*) by cleavage and (*b*) by the formation of new growing centers in the subsurface of older knobs. An account of the histology of the growing cultures is presented.

3. Though uniform in initial size and under constant cultural conditions, the cultures within each series grew at different relative rates. Thus there were differences inherent in the initial culture pieces which determined the subsequent rates of growth of the cultures.

4. Over and above the large amount of variation in growth rate of cultures started from pieces of the same size, there were differences in growth rate attributable to the initial size of the explant. The smaller the explant, the greater was the relative growth rate.

5. For each series growth curves were plotted by reference to the largest culture at any time. In these terms the cultures increased exponentially with time for 5–6 weeks. Growth rates are

described in terms of BLACKMAN's formula.

The writer is indebted to the various members of the Department of Botany for their advice and assistance during the course of the work and in the preparation of the manuscript.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BLACKMAN, V. H. The compound interest law and plant growth. Ann. Bot. **33**:353–360. 1919.
2. BUCHSBAUM, RALPH M. Size of explant and volume of medium in tissue culture. Jour. Exp. Zoöl. **63**:483–508. 1932.
3. FISHER, R. A. Some remarks on the methods formulated in a recent article on the quantitative analysis of plant growth. Ann. App. Biol. **7**:367–372. 1920.
4. GAUTHERET, R. Sur la possibilité de réaliser la culture indéfinie des tissus de tubercules de carotte. Compt. Rend. Acad. Sci. Paris **208**:118–120. 1939.
5. ———. Sur la mesure de la croissance des tissus de carotte cultivés en vitro. Compt. Rend. Acad. Sci. Paris **208**:1340. 1939.
6. ———. Sur la culture de tissus de carotte ou de topinambour, meme à l'état de lames réduite à une assize de cellules. Compt. Rend. Acad. Sci. Paris **214**:805–807. 1942.
7. ———. Titres et travaux scientifiques. Jouve et Cie, Paris. 1942.
8. HABERLANDT, G. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. Wiss. Wien, math.-naturw. Kl. **111**:69–92. 1902.
9. HILDEBRANDT, A. C.; RIKER, A. J.; and DUGGAR, B. M. Growth in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar. Amer. Jour. Bot. **32**:357–361. 1945.
10. ———; ———; and ———. The influence of the composition of the medium on growth in vitro of excised tobacco and sunflower tissue cultures. Amer. Jour. Bot. **33**:591–597. 1946.
11. KOTTE, W. Wurzelmeristem in Gewebekultur. Ber. d. bot. Ges. **40**:269–272. 1922.
12. ———. Kulturversuche mit isolierten Wurzelspitzen. Beitr. Allg. Bot. **2**:413–434. 1922.
13. NOBECOURT, P. Sur la pérennité et l'augmentation de volume des cultures de tissus végétaux. Compt. Rend. Soc. Biol. Paris **130**:1270. 1939.
14. ———. Cultures de tissus de quelques végétaux. Bull. Mens. Soc. Linnéenne Lyon **10**:83–86. 1941.
15. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. BOT. GAZ. **73**:376–390. 1922.
16. ———. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. BOT. GAZ. **74**:59–79. 1922.
17. ROBBINS, W. J., and MANEVAL, W. E. Further experiments on growth of excised root tips under sterile conditions. BOT. GAZ. **76**:274–287. 1923.
18. WHITE, P. R. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**:585–600. 1934.
19. ———. Plant tissue cultures. Bot. Rev. **2**:419–437. 1936.
20. ———. Amino acids in the nutrition of excised tomato roots. Plant Physiol. **12**:793–802. 1937.
21. ———. Glycine in the nutrition of excised tomato roots. Plant Physiol. **14**:527–538. 1939.
22. ———. Potentially unlimited growth of excised plant callus in an artificial nutrient. Amer. Jour. Bot. **26**:59–64. 1939.
23. ———. A handbook of plant tissue culture. Jaques Cattell Press, Lancaster, Pa. 1943.
24. ———. Plant tissue cultures. II. Bot. Rev. **12**:521–529. 1946.

DEVELOPMENT OF OVULE AND MEGAGAMETOPHYTE IN POMEGRANATE

J. R. KING[1]

Introduction

There appeared in 1935 a publication entitled "Development and vascular anatomy of the flower of *Punica granatum* L." by CHIH-LUNG TUNG (8). Just prior to this, there was completed in the Division of Pomology at Davis a similar study by W. B. HEWITT (unpublished). Since these two studies substantiate each other almost completely, it has been considered unwise to publish the work of HEWITT. TUNG reported, however, that the outer, or upper, whorl of carpels arises immediately after the initiation of the petal primordia and numbers from four to eight; HEWITT found that this whorl arises at about the same time as the petal primordia and numbers four to twelve. Likewise, TUNG mentioned that the innermost, or third, whorl of carpels consists of only one carpel or is, in most cases, absent. HEWITT, on the other hand, found more than one carpel in the innermost whorl, but seldom were there more than two.

Since neither of these workers investigated the development of the megagametophyte in pomegranate, such a study was made here before the war. This paper is a report of that work and serves to round out more completely the story of floral development in the pomegranate.

Investigation

The material used was collected from trees of the Wonderful variety growing near the University Farm at Davis. The ovaries were fixed in both Navashin's fluid and formalin–acetic acid–alcohol, dehydrated and cleared by the alcohol-xylol method, and imbedded in paraffin. Sections were cut 12–18 μ thick, depending upon the size of the material, and were stained with either iron alum–hematoxylin or safranin and fast green.

The ovules arise opposite vascular branches and are first indicated by the division of one to several cells in the hypodermal layer. These small groups of ovule initials are distributed indeterminately over the entire placental region of the carpel. Succeeding divisions cause a protrusion into the locule. These occurrences are substantiated by TUNG (8) and in the unpublished work on pomegranate by HEWITT.

The young ovule is projected by the short funiculus beyond the placental surface. Greater growth on one side, however, causes the ovule gradually to bend, so that at maturity it is in the anatropous position. The bending may be either to the left or to the right. Usually, however, the ovules in one half of the locule are turned in the opposite direction from those in the other half. Ovules near the center of the carpel are older than those near the outer edges.

The origin of the inner of the two integuments is first evident as a division of an epidermal cell of the ovule followed by division of adjacent epidermal cells at the same level. This ring of meristematic cells divides toward the tip of the ovule. The ovule, at the time of initiation of the inner integument, has curved until the tip is almost parallel with the placenta. The outer integument is initiated

[1] Assistant pomologist, University of California, Davis, Calif.

in the same manner and at approximately the same time as the inner, but originates four or five cells below the latter (fig. 1). As the ovule continues to develop, the greater growth of the outer integument causes it to surpass the inner.

The archesporial cell arises usually as the fourth cell beneath the epidermis soon after the integuments begin growth. It is distinguished by its large size, dense cytoplasm, and large centrally placed nucleus. The archesporial cell becomes the megaspore mother cell directly, and no parietal tissue is formed. Such a change is accompanied by increase in size and by elongation (fig. 2). Its frequent later position deeper in the nucellar tissue is brought about by division of overlying nucellar cells rather than by the formation of parietal cells.

The meiotic division of the megaspore mother cell in the formation of the two daughter cells (fig. 3) is accompanied by a slight breakdown of surrounding nucellar tissue. The second division results in a linear group of four megaspores.

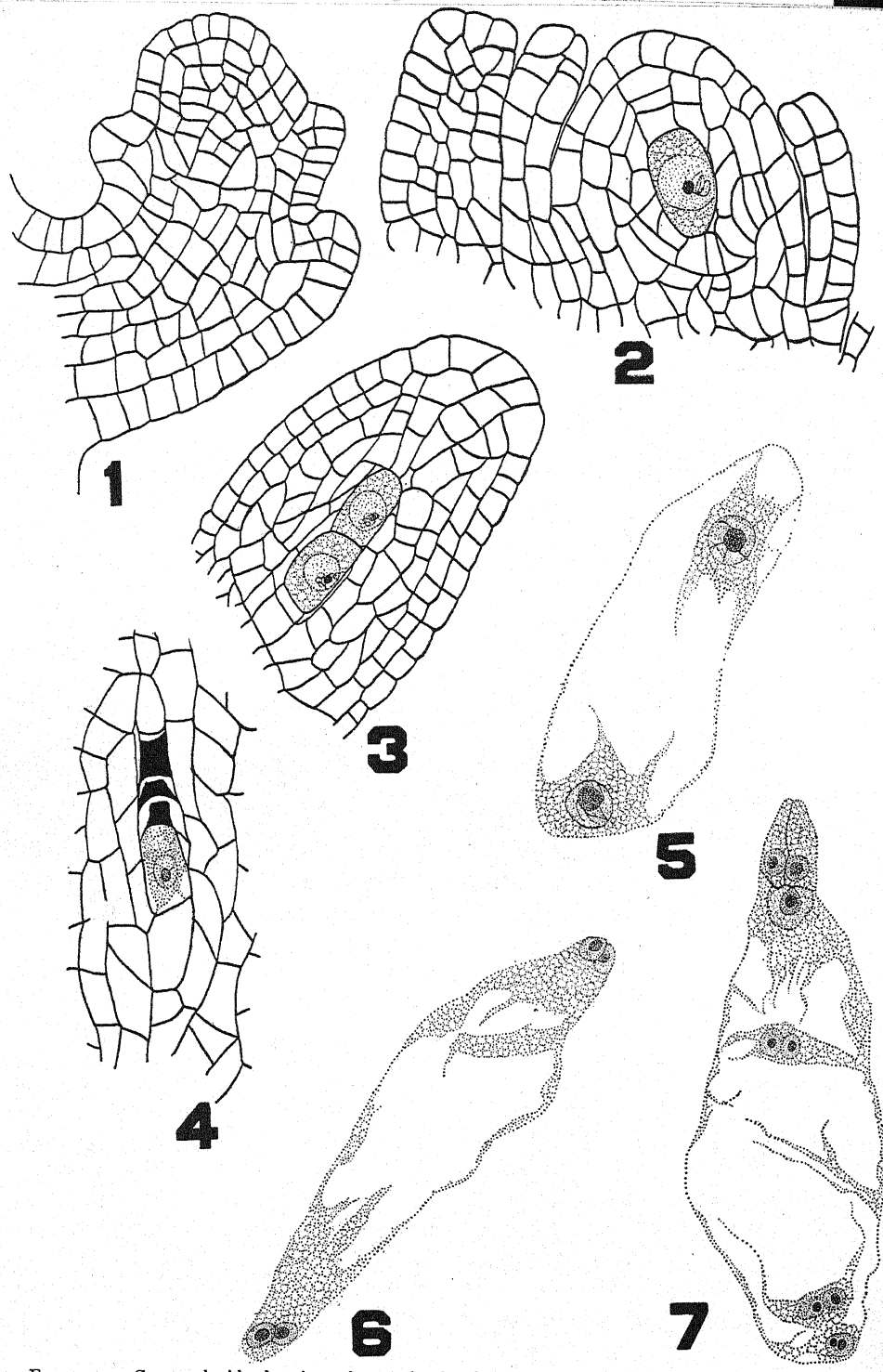
Only the chalazal megaspore takes part in the formation of the megagametophyte. The upper three megaspores are nonfunctional and gradually degenerate into dark staining, structureless masses (fig. 4). They later disappear, and their places are taken by the developing megagametophyte. Although the early changes and nuclear division of the one-nucleate megagametophyte could not be followed closely, the two-nucleate condition presents a much enlarged megagametophyte both in length and in width. The two free nuclei, one in each end of the developing megagametophyte, are separated by a large vacuole (fig. 5). The second nuclear division, which results in the four-nucleate condition, is accompanied by further increase in size of the megagametophyte and of the vacuole. The four free nuclei are usually present as a single pair in each end of the megagametophyte (fig. 6). Occasionally, however, they assume positions about equally distant from one another.

As the ovule matures, the outer integument exceeds the inner in length and width and finally envelops the ovule, except at the micropyle. The outer integument is usually from four to eight cells in width midway between the micropyle and chalaza but widens toward the micropylar end. It may in later stages separate from the nucellus and inner integument. The latter two, however, remain appressed except at the micropyle. The inner integument remains two cells wide for most of its length but broadens near the micropylar end.

The nucellus gradually disintegrates as the megagametophyte enlarges; however, when the latter is mature, nucellar tissue is still present in scattered areas.

The vascular trace, which enters the funiculus and extends through the raphe, splits into three or four branches as it nears the chalaza. Each branch diverges usually at about the same level. The main trace continues through the raphe and into the chalaza, where it may branch again.

Nuclear division in the four-nucleate megagametophyte results in a typical eight-nucleate condition, with a group of four nuclei in each end separated by a large vacuole. Early migration of one nucleus from both the micropylar and the chalazal regions toward the center of the megagametophyte is followed soon by cell-plate formation which results in a typical seven-celled, eight-nucleate megagametophyte, although the antipodal cells degenerate very early (fig. 7). The shape of the mature megagametophyte varies considerably, especially in



FIGS. 1–7.—Camera lucida drawings. Stages in development of ovule and megagametophyte. Fig. 1, developing ovule showing origin of inner and outer integuments. ×575. Fig. 2, megaspore mother cell in young ovule develops directly from archesporial cell without formation of parietal tissue. ×700. Fig. 3, two daughter cells following reduction division of megaspore mother cell. ×700. Fig. 4, three degenerating megaspores and one developing megaspore in linear arrangement. These four megaspores result from second meiotic division of spore mother cell. Developing chalazal megaspore becomes young megagametophyte. ×700. Fig. 5, two-nucleate megagametophyte. ×575. Fig. 6, four-nucleate megagametophyte. ×575. Fig. 7, seven-celled, eight-nucleate megagametophyte, consisting of two synergids, one egg, two polar nuclei, and three antipodals. Antipodal cells shown have already begun to degenerate. ×575.

width. More often it is rather broad just below the center, then tapers toward both the micropylar and the chalazal ends.

The two synergids of the egg apparatus are elongated. The nucleus of each lies in the lower portion and on the same focal plane. A conspicuous vacuole, either above or below the nucleus, was very seldom evident; however, a less dense cytoplasmic area usually does exist above the nucleus.

The egg is frequently situated slightly below and midway between the two synergids; however, this position varies. In some instances it may be found beneath either the right or the left synergid. In *Carya glabra* the synergids have occasionally been found to the left of the egg when the section is cut perpendicular to the plane of the middle septum (4). The egg is usually not distinct from the synergids in having a larger nucleus or more dense cytoplasm. A similar condition exists in *Saxifraga* (1). The egg nucleus in pomegranate varies in size. Sometimes it is larger and sometimes it is smaller than those of the synergids or polar nuclei.

The two nuclei which are to become the polar nuclei migrate early from each end of the megagametophyte and lie close to one another, usually near the center of the megagametophyte They fuse before fertilization. Frequently they are found in contact or fused near the side of the megagametophyte or in the micropylar end near the egg apparatus, as in *Lycopersicum* (2). The large nucleoli remain distinct after the cells fuse.

The true nature of the mature megagametophyte in pomegranate is rather difficult to determine because of the early beginning of degeneration of the antipodal cells. In all the mature megagametophytes observed, at least a slight degree of degeneration was apparent. The position and appearance of the antipodals vary considerably. One of the three is usually nearer the chalaza, and the group forms a triangle. The triangle may be inverted in shape, with two antipodals nearer the chalaza than the third. Occasionally the three cells are on nearly the same level. In any event, degeneration begins first in the lowest of the three cells and is soon followed by degeneration in the upper ones. The disappearance of the cell wall is an early indication of antipodal cell degeneration.

Discussion

SCHNARF (6) in 1931 stated that the embryological behavior of the Punicaceae was entirely unknown, and a survey of the literature on megagametophyte development has revealed no reference to such studies since that time. It is not possible, therefore, now to make comparisons with other members of the family. The characteristics involved in the formation of the megagametophyte of the pomegranate are those of the "normal" type, which is characterized chiefly by the fact that there occur five successive nuclear division stages in the origin of the egg cell from the megaspore mother cell and also by the fact that only one megaspore is involved in the formation of the megagametophyte. Considerable variation exists in the various phases of this type of megagametophyte development—for example, in the formation of the megagametophyte, in the divisions of the megaspore nucleus, and in the organization of the mature megagametophyte.

Discussions of the normal type of megagametophyte development and its many variations have been presented and have been ably reviewed by others (3, 5, 7).

Summary

The ovule of pomegranate is anatropous. The formation of the megagametophyte is of the "normal" type, which is characterized by the fact that there occur five successive nuclear division stages in the origin of the egg cell from the megaspore mother cell and also by the fact that only one megaspore is involved in the formation of the eight-nucleate megagametophyte.

DIVISION OF POMOLOGY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

LITERATURE CITED

1. CHAPMAN, M. The ovule and embryo sac of *Saxifraga virginiensis*. Amer. Jour. Bot. 20: 151–158. 1933.
2. COOPER, D. C. Macrosporogenesis and the development of the macrogametophyte of *Lycopersicon esculentum*. Amer. Jour. Bot. 18:737–748. 1931.
3. COULTER, J. M. Relation of megaspores to embryo sacs in angiosperms. BOT. GAZ. 45:361–366. 1908.
4. LANGDON, L. M. Embryogeny of *Carya* and *Juglans*, a comparative study. BOT. GAZ. 96:93–117. 1934.
5. MAHESHWARI, P. A critical review of the types of embryo sacs in angiosperms. New Phytol. 36: 359–417. 1937.
6. SCHNARF, K. Vergleichende Embryologie der Angiospermen. Berlin. 1931.
7. ———. Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. 2:565–585. 1936.
8. TUNG, CHIH-LUNG. Development and vascular anatomy of the flower of *Punica granatum* L. Bull. Chinese Bot. Soc. 1:108–118. 1935.

ETHER-SOLUBLE ORGANIC ACIDS AND BUFFER PROPERTIES OF CITRUS PEELS[1]

WALTON B. SINCLAIR AND DESIRE M. ENY

Introduction

The fact that the pH of the peel of citrus fruits (pH 5.0, or greater) is much higher than that of the corresponding juices of the pulp (10, 11, 12) indicates that the buffer properties of the two systems are quite different. Owing to the high pH, the organic acids present in the peel exist chiefly in the salt form and not in the free state. Furthermore, the high pH values of the peel strongly indicate that the buffer system is composed of salts of inorganic acids and not of organic acids and their salts.

Previous investigations (13, 14) have shown that the buffer system of citrus juices is composed of organic acids and their salts and that these juices are similar to pure citric acid solutions containing equivalent amounts of free acid and salt of a strong base with a common anion. No positive statement can be made with respect to the buffer system of the peel. So far as the authors know, no thorough study of the buffer systems of citrus peels has been made. Such data are essential, however, for the understanding of certain physiological relations between peel and pulp and for the understanding of the metabolic reactions of the peel under storage conditions.

The present studies were carried out to determine what constituents of the peel of oranges, grapefruit, and lemons

[1] Paper no. 553, University of California Citrus Experiment Station, Riverside, Calif.

are responsible for the buffer capacities. The experiments involved the determination of the ether-soluble organic acids both in the extract of the fresh peel and in the dried peel. The organic acids (citric, malic, and oxalic) of the peel extract were determined both on a lead acetate precipitate and on an ether extract. Titration curves for the peel extract of oranges, grapefruit, and lemons are presented so that a comparison can be made between the buffer properties and the organic acids that occur in the peel. From these data, the relation of pH to the free and the combined acids can be determined.

Material and methods

Preparation of samples

The samples (fifty to one hundred fruits each) of mature navel and Valencia oranges, grapefruit, and lemons were taken from plots of trees located in experimental orchards at the University of California Citrus Experiment Station and in commercial groves in the vicinity of Riverside, California. The peel was carefully removed from the fruit to avoid contamination with the juice. As the juice of citrus fruits is high in organic acids, contamination of the peel with a small amount of juice from the vesicles of the pulp would increase excessively the total acid content of peel samples.

The peel extract was prepared by cutting the fresh peel into small pieces, placing them in glass jars with covers, and then freezing for 48–60 hours at $-25°$ C. After thawing, the peel was quickly ground through a galvanized meat-chopper. The juice was extracted from this ground mass by means of a hydraulic press, at a pressure of 20,000 lb./sq. in. The liquid obtained will be referred to in the remainder of this paper as the peel "sap."

The dried-peel samples were obtained by taking aliquot portions of the ground peel, and of other peel samples that were not frozen, and drying them in a mechanically ventilated oven at 65° C. The dried peel was prepared for the analyses by grinding to a fine powder in a Wiley mill.

For moisture determinations, known amounts of fresh peel were weighed in small aluminum containers.

Chemical methods

Preliminary experiments early showed that the individual organic acids could not be determined accurately on the peel sap in the presence of the large concentration of pectin but had to be determined on a pectin-free extract. To precipitate the pectins, sufficient ethyl alcohol was added to a known volume of sap to produce a final concentration of 85% alcohol, and the precipitate was separated from the alcoholic solution by either filtering or centrifuging.

Ether liquid extraction.—In the use of this alcoholic solution for the isolation of the organic acids with ether by the liquid extraction method, the alcohol was completely driven off on a steam bath without allowing the solution to become dry. This was done by evaporating to a very low volume, adding distilled water frequently, and re-evaporating until the solution was free of alcohol. The resulting solution, free of both pectin and alcohol, was diluted with distilled water to the original volume of the sap. It was then subjected to a continuous ethyl ether liquid extraction in a Bailey-Walker apparatus modified to include a small funnel and a glass thimble to hold the liquid, instead of the usual syphon cup. As the funnel rested on the bottom of the thimble, the condensed ether passed through the column

of the liquid sample, and complete extraction occurred in between 24 and 30 hours. A small amount of water was then added to the ether extract, and all the ether was carefully evaporated off, leaving the organic acids in the aqueous phase. This liquid was filtered and subsequently diluted to a known volume. Aliquots were used for determining the ether-soluble organic acids in the peel sap.

LEAD ACETATE PRECIPITATION.—In supplementary comparative determinations, the organic acids were precipitated by lead acetate from the pectin-free alcoholic solution (85%) referred to earlier, according to the procedure of HARTMANN and HILLIG (3). The precipitate was washed with alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulfide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. Aliquots of this solution were used for determining the organic acids in the peel sap which had been precipitated by lead acetate.

EXTRACTION OF DRIED PEEL.—Organic acids were isolated from dried peel by the method of PUCHER *et al.* (6). A known weight of ground dried peel was acidified to pH 1 with sulfuric acid (4*N*), mixed thoroughly with asbestos, and subsequently extracted with absolute ether (H_2O_2-free) for 30 hours. Water was added to the extract, and the ether was carefully evaporated from the solution, leaving the organic acids in the aqueous phase. This solution was filtered into a volumetric flask and diluted to final volume. Aliquots were used for the analysis of the organic acids.

DETERMINATION OF ORGANIC ACIDS.—With a Beckman glass-electrode pH meter, the total organic acids were determined by titrating the extracts between the limits of pH 7.8 and 2.6, according to the method of VAN SLYKE and PALMER (15). Since oxalic acid reacted as a monobasic acid under these conditions, a correction was made by determining independently the amount of oxalic acid in the samples. Suitable aliquots, depending upon the oxalic acid content, were measured into 100-ml. beakers and acidified to Congo red with 0.5*N* HCl. The material that flocculated on standing was filtered off on a fine-sintered crucible and washed with water. The precipitation and titration of the oxalate from this clear liquid were carried out according to the procedure of PUCHER *et al.* (6).

Quantitative determination of citric acid, free and combined, was made by the pentabromacetone method of PUCHER *et al.* (7). The sample was heated with H_2SO_4 to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by $KMnO_4$ in the presence of KBr. After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S and was subsequently titrated with standard $AgNO_3$. The citric acid in the original sample was calculated from the titration.

Malic acid was also determined by the pentabromacetone method of PUCHER *et al.* (7). The principle of the method involves the oxidation of malic acid with $KMnO_4$, in the presence of KBr, to a bromine compound volatile with steam. This compound reacts with 2,4-dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. The pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

Titration curves of both normal and pectin-free peel sap were determined at 23° C. with a Beckman glass-electrode pH meter.

Results

Organic acids of peel sap, determined by the ether liquid extraction method

The data in table 1 show the amounts of the various organic acids obtained from peel sap by continuous extraction with absolute ether. The relatively high pH values of the sap indicate that 75–80% of the organic acids are in the salt form. As the extraction was performed at pH 1, the ether extract of the sap included, in the free form, the total organic acid radicals. The values in column 5 of table 1 show the milliequivalents per milliliter (me./ml.) of organic acids, or the total organic acid, extracted from the sap with ether and subsequently determined by titration between pH 7.8–2.6, with corrections for oxalic acid and other variables. The amounts of organic acids in the peel saps of citrus fruits are very low in comparison with those in the juices of the pulp (11, 12).

The total polybasic acids extracted from the peel saps with ether are represented chiefly by citric and malic acids (table 1). A trace of oxalic acid was present. The relatively high pH values of the saps indicated that the oxalic acid existed chiefly as an insoluble salt and did not occur in appreciable concentrations in the centrifuged saps. The sum of the milliequivalents of citric and malic acids agreed satisfactorily with the total organic acids (milliequivalents) titrated between pH 7.8 and 2.6. This is good evidence that if other nonvolatile organic acids were extracted with ether, they were present in the sap in only microquantities. In the peel sap the malic acid concentration is much higher than that of citric acid; in citrus juices, the reverse order prevails.

Organic acids of peel sap precipitated with lead acetate

The completeness with which lead acetate precipitated the organic acids and acid salts is made evident by the close agreement of the sum of the milliequivalents of citric and malic acids with the total-acid values (titrated between pH 7.8 and 2.6) of the ether extract (table 1). The citric and malic acid values of the ether extract agree substantially with similar values of the lead-acetate precipitate. The close agreement of the values determined by these two entirely different methods indicates, to an accuracy of 5%, the actual organic acid content of the peel sap. The conclusion can also be drawn that the liquid extraction method is satisfactory for the isolation of organic acids from citrus-peel saps.

Ether-soluble organic acids of dried citrus peels

The data in table 2 report the amounts of organic acids (mg./gm. fresh weight) in the peels of navel and Valencia oranges, lemon, and grapefruit. Although data are reported on a fresh-weight basis, actual determinations were made on the ether extract of dried peel. In these determinations the organic acid content was greatest in navel orange peel and least in grapefruit. The concentrations of total acids in the peels of Valencia orange and lemon fell between these two extremes. Although the relatively larger amounts of organic acids in navel orange peel cannot be explained, it should be mentioned that these peel samples were more mature than the others. The smaller amount of organic

TABLE 1

ORGANIC ACIDS OF PEEL SAP OF CITRUS FRUITS, DETERMINED BY LIQUID EXTRACTION AND LEAD ACETATE PRECIPITATION METHODS

Sample no. and variety	Sampling date (1946)	pH of sap	Free acid of pectin-free sap	Ether-soluble organic acids						Acid constituents precipitated by lead acetate				
				Total organic acids (pH 7.8–2.6)	Citric acid		Malic acid		Total citric and malic acids	Citric acid		Malic acid		Total citric and malic acids
			me./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	me./ml.
1. Navel orange	Feb. 26	5.00	0.022	0.052	1.22	0.019	2.35	0.035	0.054	1.54	0.024	2.28	0.034	0.058
2. Navel orange	Mar. 11	5.05	.024	.055	0.58	.009	2.82	.042	.051	0.38	.006	3.08	.046	.052
3. Navel orange	Apr. 2	5.40	.015	.029	0.38	.006	1.41	.021	.027	0.32	.005	1.34	.020	.025
4. Lemon	Apr. 17	5.38	.015	.020	0.32	.005	1.01	.015	.020	0.32	.005	1.01	.015	.020
5. Lemon	Apr. 27	5.48	.014	.020	0.19	.003	0.94	.014	.017	0.26	.004	1.01	.015	.019
6. Grapefruit	May 26	5.70	.015	.028	0.38	.006	1.34	.020	.026	0.38	.006	1.41	.021	.027
7. Valencia orange	June 4	5.25	0.022	0.038	0.26	0.004	2.08	0.031	0.035	0.26	0.004	2.14	0.032	0.036

TABLE 2

ETHER-SOLUBLE ORGANIC ACIDS OF DRIED PEEL OF DIFFERENT VARIETIES OF CITRUS

SAMPLE NO. AND VARIETY	MOISTURE (%)	pH OF SAP	ETHER-SOLUBLE ORGANIC ACIDS									
			Total acids, as citric (pH 7.8–2.6)		Citric acid		Malic acid		Oxalic acid		Total citric, malic, and oxalic acids	
			mg./gm.*	me./gm.†	mg./gm.	me./gm.	mg./gm.	me./gm.	mg./gm.	me./gm.	mg./gm.*	me./gm.
1. Navel orange	73.02	5.00	4.93	0.077	0.38	0.006	2.61	0.039	1.13	0.025	4.48	0.070
2. Navel orange	73.51	5.05	5.12	.080	.51	.008	2.88	.043	1.26	.028	5.06	.079
3. Navel orange	76.07	5.40	5.32	.083	.51	.008	2.21	.033	1.80	.040	5.19	.081
4. Navel orange	74.80	5.30	3.07	.048	.32	.005	1.41	.021	0.95	.021	3.01	.047
5. Valencia orange	77.24	‡	3.14	.049	.26	.004	1.61	.024	0.90	.020	3.07	.048
6. Valencia orange	74.95	‡	3.33	.052	.32	.005	1.54	.023	1.08	.024	3.33	.052
7. Valencia orange	73.56	‡	3.97	.062	.32	.005	2.28	.034	0.99	.022	3.91	.061
8. Valencia orange	75.11	5.25	3.84	.060	.32	.005	2.01	.030	1.08	.024	3.78	.059
9. Grapefruit	80.18	‡	2.88	.045	.32	.005	1.27	.019	0.86	.019	2.75	.043
10. Grapefruit	80.56	‡	2.75	.043	.32	.005	1.47	.022	0.68	.015	2.69	.042
11. Grapefruit	82.39	‡	2.37	.037	.38	.006	1.34	.020	0.50	.011	2.37	.037
12. Grapefruit	81.52	5.65	2.88	.045	.38	.006	1.68	.025	0.59	.013	2.82	.044
13. Lemon	83.59	5.38	3.59	.056	.26	.004	1.27	.019	1.40	.031	3.46	.054
14. Lemon	83.54	5.48	3.14	.049	.38	.006	1.07	.016	1.40	.031	3.39	.053
15. Lemon	84.24	5.53	2.18	.034	.32	.005	0.94	.014	0.63	.014	2.11	.033
16. Lemon	85.04	‡	2.88	.045	.32	.005	1.34	.020	0.86	.019	2.82	.044
17. Lemon	83.92	‡	2.43	0.038	0.19	0.003	1.27	0.019	0.63	0.014	2.31	0.036

* Milligrams of acid (as citric) per gram of fresh peel.

† Milliequivalent per gram of fresh peel.

‡ Data undetermined.

acids in grapefruit peel is reflected in the higher pH of its peel sap.

The distinctive features of table 2 are the comparatively low concentration of citric acids and the relatively high concentrations of malic and oxalic acids in the various peel samples. By extracting dried peel with ether at pH 1, the insoluble oxalates were made soluble and were readily removed from the tissue by the solvent. The abundance of calcium in the peel (table 3) indicates that any oxalic acid occurring in the peel would be immediately converted to the insoluble calcium salt. This is, of course, true only if an excess of calcium is present in the peel over that required to form calcium pectate. The fact that only a trace of oxalic acid appeared in the peel sap is evidence that sufficient calcium was present to convert the oxalic acid to insoluble calcium oxalate. The high pH of the peel sap is also evidence that the oxalic acid is in the salt form. These data substantiate the observations of BARTHOLOMEW and REED (2), who have noted the presence of calcium oxalate crystals in the peels of citrus fruits; and of PIERCE and APPLEMAN (5), who have shown that plants high in oxalic acid contained only traces of sap-soluble calcium. The high calcium content of peel (table 3), in comparison with that of pulp and juice, is certainly highly correlated with the amount of insoluble oxalate. In fact, calcium and magnesium are the only inorganic cations which exist in the peel in concentrations sufficiently high to form crystalline insoluble salts with the organic acids.

Calcium in the peel may react with other chemical substances in the sap besides oxalic acid. As suggested previously, amorphous precipitates such as calcium pectate may occur as the result of reaction of calcium with pectin. Undoubtedly, the inorganic cations precipitate, at various rates, the hydrated colloids of the peel. A protective action is exerted by many organic colloids to prevent the precipitation of compounds that have exceeded their solubility product in pure solution.

The exceptionally low concentration of organic acids in the peel of citrus fruits, in comparison with that in the pulp, strongly suggests that the location of organic acid synthesis is in the pulp in citrus fruits, according to the following chain of reasoning: Citrus fruits, unlike pomaceous fruits, have no fleshy centers that are permeated by vascular bundles. The vascular system is chiefly confined to the mesocarp (spongy parenchyma of the peel), which therefore serves the important function of trans-

TABLE 3

RELATIVE AMOUNTS OF CALCIUM AND MAGNESIUM IN PEEL, PULP, AND JUICE OF ORANGES AND GRAPEFRUIT

VARIETY	MOISTURE (% FRESH WEIGHT)			CALCIUM (% DRY WEIGHT)			MAGNESIUM (% DRY WEIGHT)		
	Peel	Pulp	Juice	Peel	Pulp	Juice	Peel	Pulp	Juice
Valencia orange	71.0	84.9	87.2	0.76	0.30	0.06	0.12	0.08	0.09
Navel orange..	74.0	85.7	88.1	.56	.31	.06	.10	.09	.11
Grapefruit.....	80.3	87.3	89.1	0.64	0.26	0.07	0.10	0.10	0.11

porting water and solutes from the tree to the pulp vesicles. The experiments of HODGSON (4) and of BARTHOLOMEW (1) showed the existence of an equilibrium between the water in citrus trees and their fruits. This equilibrium is manifested by the diurnal changes in volume of fruits on trees subjected to an environment conducive to excessive transpiration from leaves. The results of these studies led REED (8) to conclude that the translocation of liquids in citrus fruits is through the layers of hydrophilic colloids on the cell walls of the mesocarp and that the juice vesicles in the pulp exert sufficient suction pressure to pull water from the hydrated colloids.

If the organic acids are synthesized in the leaves and subsequently transferred to the juice vesicles through the albedo (mesocarp), it would appear that higher concentrations of these acids than are present should occur in the mesocarp. The latter contains sufficient cations to form salts (soluble and insoluble) if the organic acids pass through the peel to the vesicles, which contain large amounts of organic acids. On the contrary, however, the mesocarp is notably low in amounts of free and combined organic acids. During the entire growth and maturation of the fruit, the passage of water and solutes from leaves to vesicles, and from vesicles to leaves during excessive leaf transpiration, did not result in accumulation of organic acids in the peel of mature fruits. The lack of accumulation of organic acids under these conditions is highly suggestive that the organic acids are synthesized in the vesicles and not in the leaves. This deduction agrees with the limited experiments of RICEVUTO (9), who concluded that citric acid is formed in lemon fruits from reducing sugars and pentosans by enzymatic action.

TITRATION CURVES OF PEEL SAPS

The relation of pH to the acid constituents of peel sap can be further elucidated by following the resistance to changes in pH upon addition of increments of standard acid or alkali to a known amount of sap. Peel sap, like all plant extracts, is to a varying degree capable of resisting changes in pH upon the addition of strong acids or bases. It was therefore decided to determine experimentally what substances were responsible for the buffer effect of the sap.

It was important to determine the effect of the soluble pectins in the centrifuged sap on the shapes of the buffer curves. The titration curves of normal sap (curves *A*) and of sap from which pectins had been removed (curves *B*) are shown for navel and Valencia oranges, grapefruit, and lemons in figures 1–4, respectively. Although the two curves in each figure are nearly parallel, they are distinctly different. The alcohol used for precipitating pectins probably precipitated some of the soluble buffer salts also and thus caused an increase in acidity and lowered the position of curve *B* on the graph. The amounts of free acid in these saps are represented by those portions of the curves between zero and the milliequivalents of NaOH required to bring the system to a pH of 8.2. It can be seen that the concentration of free acid, organic and inorganic, is relatively small in these saps. The total-acid radical calculated from the titration curves between pH 7.8 and 2.6 was greater than the values determined from similar titrations of the ether-soluble organic acids extracted from the sap.

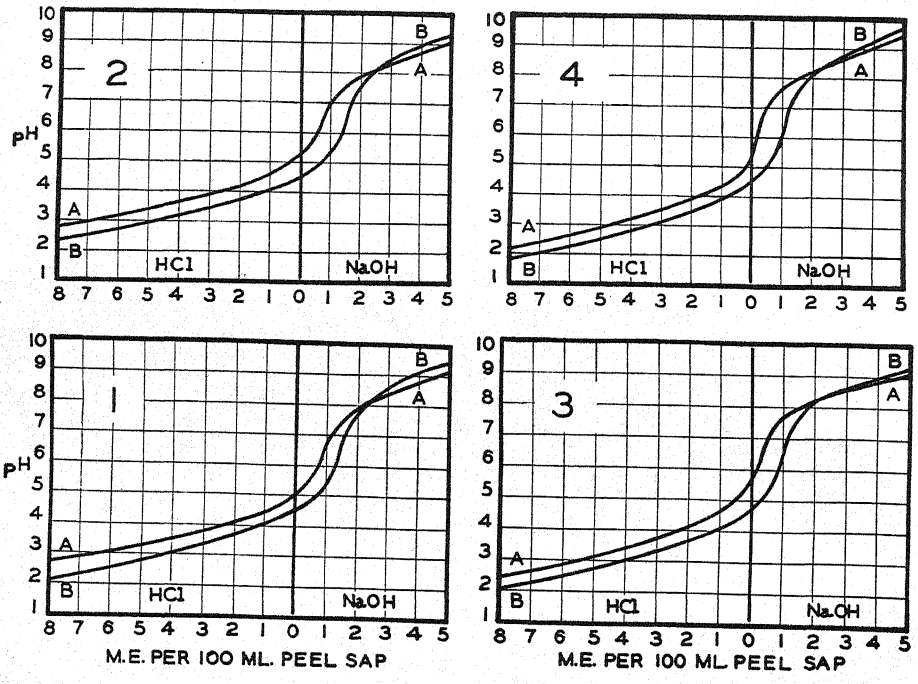
The position of the curves on the graphs, with respect to buffer properties, is influenced by the concentration of free acid in the sap. In other words, the buffer capacity of a solution is governed by the

concentration of the components. As citrus-peel sap is not highly buffered, titration curves of saps of different dilutions would have different slopes. Titration curves of peel saps of different varieties of citrus are very similar, and all of them are located at nearly the same position on the graphs. As shown by the zero points on curves *A* and *B* (figs. 3, 4), the pH of the natural saps from grapefruit and lemon peels are somewhat higher than corresponding values for peels of navel and Valencia oranges.

The titration curves of the various peel saps are vastly different from those of the corresponding juices of the pulp. The peel of citrus fruits contains a comparatively small amount of organic acids and their salts. In the absence of these constituents, the pH values of the peels are high, and these systems consequently have only a slight buffer capacity. The pulp juices of citrus fruits, on the other hand, contain larger amounts of organic acids and their salts and have lower pH values and higher buffer capacities.

Summary

1. The concentrations of ether-soluble organic acids are much lower in peels of citrus fruits (oranges, grapefruit, and lemons) than in juice samples of corresponding pulps. The high pH values of the peel saps indicate that most of the organic acids are present in the salt form and not in the free state. The amounts of citric and malic acids in the expressed saps agreed satisfactorily with the total organic acids titrated between pH 7.8 and 2.6. The organic acids precipitated from the peel sap with lead acetate



FIGS. 1–4.—Titration curves of peel sap. *A*, normal; *B*, pectin removed. Fig. 1, navel orange. Fig. 2, Valencia orange. Fig. 3, grapefruit. Fig. 4, lemon.

agreed with the total organic acids titrated between pH 7.8 and 2.6 on a water solution of the ether extract. The citric and malic acids values of the ether extract agreed substantially with similar values of the lead acetate precipitate. The ether liquid extraction method is satisfactory for the isolation of organic acids from citrus-peel saps.

2. The total nonvolatile organic acids were extracted from dried peel of citrus fruits with absolute ether at pH 1. The organic acid content consisted chiefly of citric, malic, and oxalic acids. The citric acid content is very low in comparison with the concentrations of malic and oxalic acids. The oxalic acid is present in the peel as insoluble calcium oxalate, with the exception of a trace occurring in the peel sap.

3. The titration curves of peel saps of citrus fruits (navel and Valencia oranges, grapefruit, and lemons) show that these systems contain comparatively small amounts of organic acids and their salts. In the absence of these constituents, the pH values of the peels are high, and these systems consequently have only a slight buffer capacity.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BARTHOLOMEW, E. T. Internal decline of lemons. III. Water deficit in lemon fruits caused by excessive leaf evaporation. Amer. Jour. Bot. 13:102–117. 1926.
2. BARTHOLOMEW, E. T., and REED, H. S. General morphology, histology, and physiology. Chap. vi *in* H. J. WEBBER and L. D. BATCHELOR (eds.). The citrus industry. 1:669–717. University of California Press, Berkeley. 1943.
3. HARTMANN, B. G., and HILLIG, F. Acid constituents of food products: special reference to citric, malic, and tartaric acids. Assoc. Off. Agr. Chem. Jour. 17:522–531. 1934.
4. HODGSON, R. W. Some abnormal water relations in citrus trees of the arid Southwest and their possible significance. Univ. California Publ. Agr. Sci. 3:37–54. 1917.
5. PIERCE, E. C., and APPLEMAN, C. O. Role of ether soluble organic acids in the cation-anion balance in plants. Plant Physiol. 18:224–238. 1943.
6. PUCHER, G. W.; VICKERY, H. B.; and WAKEMAN, A. J. Determination of the acids of plant tissue. II. Total organic acids of tobacco leaf. Ind. Eng. Chem., Anal. Ed. 6:140–143. 1934.
7. ———. Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acids. Ind. Eng. Chem., Anal. Ed. 6:288–291. 1934.
8. REED, H. S. The swelling of citrus fruits. Amer. Jour Bot. 17:971–982. 1930.
9. RICEVUTO, A. The formation of citric acid in lemons. Ann. di Chim. Appli. (Rome) 23:411–413. 1933. (Abst. in Chem. Abs. 28:1072. 1934.)
10. SINCLAIR, W. B.; BARTHOLOMEW, E. T.; and RAMSEY, R. C. Analysis of the organic acids of orange juice. Plant Physiol. 20:3–18. 1945.
11. SINCLAIR, W. B., and ENY, D. M. The organic acids of lemon fruits. BOT. GAZ. 107:231–242. 1945.
12. ———. The organic acids of grapefruit juice. Plant Physiol. 21:140–147. 1946.
13. ———. Significance of the alkaline ash of citrus juices. Proc. Amer. Soc. Hort. Sci. 47:119–122. 1946.
14. ———. Stability of the buffer system of lemon juice. Plant Physiol. 21:522–532. 1946.
15. VAN SLYKE, D. D., and PALMER, W. W. Studies of acidosis. XVI. The titration of organic acids in urine. Jour. Biol. Chem. 41:567–585. 1920.

SURVEY OF HIGHER PLANTS FOR PRESENCE OF ANTIBACTERIAL SUBSTANCES

LESLEY E. HAYES

Introduction

The fact that substances with antibacterial properties have been found in a number of species of nongreen plants led to the supposition that similar substances occur in green plants. Although a large number of species of higher plants have been investigated for the presence of such compounds by previous workers, their work by no means exhausted the possibilities in this line of research.

Demonstration by *in vitro* methods of antibacterial properties in a substance synthesized by plants does not prove that compound to be of therapeutic value. However, *in vitro* techniques do provide a rapid method for detecting antibacterial substances which may warrant more intensive investigation. It was the purpose of this investigation to make *in vitro* tests for antibacterial activity of extracts of tissues of higher plants common to Ohio.

Review of literature

OSBORN (8), an English worker, has reported the results of an experimental survey on 2300 species of higher plants and has shown that several species in the Ranunculaceae, Cruciferae, Compositae, and Liliaceae contain substances that inhibit growth of *Staphylococcus aureus* and *Escherichia coli*.

PRATT *et al.* (9) obtained an antibiotic—chlorellin—from the culture solution and the cell mass of cultures of *Chlorella vulgaris* and *C. pyrenoidosa*. In solutions of 0.03–0.1 mg. chlorellin per cc. of water, the antibiotic caused a zone of inhibition 45 mm. wide on *S. aureus* plates.

HEATLEY (6), another English worker, reported a comprehensive study of crepin, an antibiotic obtained from the buds and flowers of *Crepis taraxacifolia*. The substance is thermostable, is soluble in pyridine and alcohol, and has a molecular weight between 236 and 275. The suggested molecular formula for crepin is $C_{14}H_{16}O_4$. It inhibits growth of certain gram-positive and gram-negative bacteria, and its inhibitory activity is enhanced by treatment with human serum for 24 hours at 37° C. It is slightly toxic to leucocytes.

SANDERS *et al.* (12), working in Indiana, tested 120 plant species, 22 of which showed varying degrees of bacterial inhibition. Juice was expressed, without use of solvents, in a Carver hydraulic press and tested against *S. aureus* and *Bacillus subtilis*.

SEEGAL and HOLDEN (13), at the University of Illinois, described activity of extracts of *Anemone pulsatilla* and a species of buttercup (name not given) against *Mycobacterium tuberculosis hominis* and *Monilia albicans*. They prepared the samples in two ways, either by steam distillation or by autoclaving an extract made from one part water to one part ground plant material. The extract was found to be toxic to laboratory animals, but the report did not state whether the extract was administered orally or parenterally.

In China, CHEN *et al.* (4) found a principle in *Eleocharis tuberosa* which was active against *S. aureus*, *Escherichia coli*,

and *B. subtilis*. This substance, which they called puchiin, is thermolabile and is soluble in water but not in ether, petroleum ether, chloroform, or benzene.

LUCAS and LEWIS (7), working in Michigan, reported antibacterial activity of water extracts of *Lonicera tartarica*, *Vaccinium corymbosum*, *Sorbus americana*, *Verbascum thapsus*, and *Paeonia officinalis*. The writer's tests on *P. officinalis* showed stimulation of bacterial growth rather than inhibition.

CAVALLITO *et al.* (2, 3) determined methods of isolation, physical properties, and antibacterial action of inhibitors found in *Arctium minus* and *Allium sativum*.

Investigation of antibiotic activity of higher plants has not been limited to antibacterial effects. TOKIN (10), a Russian worker, reported discovery of "phytoncides," the name applied to volatile substances, produced by plants, which are strongly bactericidal and protistocidal. These vapors are also lethal to yeast and to eggs of mollusks and frogs. Phytoncides have been found in roots of onion, garlic, and *Paeonia anomalia*. TOROPTSEV and FILATOVA (11), also from Russia, reported that the vapors of onion and garlic were effective in stimulating regeneration of tissue in both aseptic and infected wounds.

Material and methods

Tests were made on 231 plant species collected in Franklin, Hocking, Scioto, Pike, and Adams counties, Ohio. As a rule the determinations were carried out immediately or within 24 hours. When not used immediately, the specimens were kept wrapped in moist paper at 8° C. A few species previously tested by other workers were included because of the premise that antibacterial activity of plant extracts may vary with stage in growth of the plant or with method of testing employed. Certain species tested —ginseng, heal-all, joe-pye weed, and ninebark—were of interest because folklore attributes medicinal value to them.

GRAY (5) was used as the authority in nomenclature of species except for the Bryophytes, some of the Pteridophytes, and a few horticultural varieties.

The extracts were made by grinding the specimens in a meat-chopper and then macerating them in a Waring Blendor with from one-half to three times their weight of distilled water for 5 minutes. The amount of water added depended on the succulence of the plant. The macerated material was then filtered in a Buchner funnel under suction, using two disks of no. 1 filter paper.

The activity of plant extracts was tested by a method similar to that devised by ABRAHAM *et al.* (1) in connection with penicillin assay. Tryptose-dextrose agar was used as the culture medium in the tests. The medium was made according to the following formula:

Bacto-tryptose	20.0 gm.
Bacto-dextrose	2.0 gm.
Di-sodium phosphate	2.5 gm.
Sodium chloride	5.0 gm.
Powdered agar	15.0 gm.
Distilled water	1000.0 ml.

Plates were poured to a depth of approximately 3 mm. of agar, and care was taken to keep the plates on a level surface until the agar had solidified.

Nutrient broth, used as an inoculating medium, was prepared as follows:

Beef extract	3 gm.
Peptone	5 gm.
Sodium chloride	5 gm.
Distilled water	1000 ml.

The test organisms used were *Staphylococcus aureus*, *Escherichia coli*, *Erwinia carotovora*, and *Phytomonas tumefaciens*. The first two organisms are

TABLE 1

RESULTS OF TESTS FOR ANTIBACTERIAL ACTION OF EXTRACTS OF EIGHTEEN SPECIES OF GREEN PLANTS

.... = No activity
S = Marked stimulation
+ = Inhibitory zone, 5 mm.
++ = Inhibitory zone, 6–10 mm.
+++ = Inhibitory zone, 11–15 mm.
++++ = Inhibitory zone, 16–25 mm.
+++++ = Inhibitory zone, 26–50 mm.

Species	Staphylococcus aureus	Escherichia coli	Erwinia carotovora	Phytomonas tumefaciens
Allium cernuum				
Leaves	++++	+	++	+++
Leaf, stem, flower	S	....	+	....
Bulbs	S	....	....	++
A. tricoccum				
Leaves	++++	++	++++	++++
A. vineale				
Leaf	+++	++++	+++	+++
Leaf, stem, flower	SS	S	+	....
Bulbs	++++	+	++	++++
Bulbs (2d sample)	++++	+	++	++++
Asarum canadense				
Leaf, stem, flower	++++	....	....	....
Leaf, stem, root	++	....	....	....
Leaf, stem, root (boiled 5 min.)	++	....	....	....
Barbarea vulgaris				
Winter rosette	++	S	+	++
Entire plant (summer)	S	S	S	S
Summer rosette	....	....	....	....
Berberis thunbergii				
Fruit	+++	+++	++	+
Fruit (2d sample)	+++	+++	++	+
Flowers	S	S	+	....
Celastrus scandens				
Fruit	+	+++	++	+
Convolvulus arvensis				
Leaf, stem	....	....	++++	....
Root	....	....	++++	++
Leaf, stem	....	....	++++	?
Crataegus sp.				
Fruit	++	+	+	+
Erythronium americanum				
Leaves, flowers	+	+	++	....
Ilex decidua				
Fruit	+	++	+++	....
Fruit (2d sample)	+	++	+++	....
Lepidium draba				
All but root	+++	+++	+	+
Entire plant	++	++	+	++
Medeola virginiana				
Leaf, stem, root	+	....	++	S

TABLE 1—*Continued*

Species	Staphylococcus aureus	Escherichia coli	Erwinia carotovora	Phytomonas tumefaciens
Oenothera biennis				
Winter rosette	.....	+++	.....	.....
Summer leaf, stem, root	.....	.....	.....	.....
Ranunculus abortivus				
All but root	+++	.....	+++	.....
Entire plant	+++++	+++++	.....	.....
Entire plant (2d sample)	+++++	+++++	.....	.....
Sanguinaria canadensis				
Entire plant	+++	S	.....	.....
Leaf, stem, rhizome	++	.....	.....	.....
Leaf, stem, rhizome (extract boiled 5 min.)	++	.....	.....	.....
Dried rhizome	++	.....	.....	.....
Taxus canadensis				
Fruit	+++	+++	++++	.....
Fruit (boiled 5 min.)	+++	+++	++++	.....
Leaves	S	.....	S	.....
Verbascum thapsus				
Leaves (after flowering)	.....	.....	.....	.....
Spring rosette	.....	.....	++	+
Spring rosette and root	.....	.....	++	+

sometimes pathogenic to man, and the last two are pathogenic to plants. *S. aureus* is gram-positive, the other three are gram-negative.

The agar plates were inoculated by flooding with 24-hour nutrient-broth cultures of the test organism and allowed to drain inverted on a flat board sterilized with phenol solution.

Sterile cylinders (made from 10 mm. lengths of glass tubing, 15 mm. in diameter, with one end beveled toward the inside) were placed on the inoculated agar with slight pressure, making a water- and bacteria-tight seal. Approximately 0.5 ml. of extract to be tested was introduced into this cylinder. Throughout the procedure the usual precautions were taken to prevent contamination. One to six samples per plate could be tested. Plates of *Staphylococcus aureus* and *Escherichia coli* were incubated 16–18 hours at 37° C., and plates of the plant pathogens were incubated 18–24 hours at room temperature (approximately 27° C.). The tests were carried out in triplicate, and results were evaluated by measuring zones of inhibition or stimulation of bacterial growth.

Results and discussion

Some of the extracts markedly inhibited bacterial growth, some caused increased bacterial growth, and others showed no activity. Of the 231 species tested, extracts from 46 inhibited growth of one or more species of the test organisms. The most marked inhibition was obtained in the tests of the species listed in table 1.

The following 213 species showed little or no inhibition of bacterial growth:

Acanthopyrus sp.
Achillea millefolium
Acorus calamus
Adiantum pedatum
Aesculus hippocastanum
Agrimonia sp.
Agrostemma githago
Ambrosia artemisiifolia
A. trifida

Anemone canadensis
Antennaria parlinii
A. plantaginifolia
Apocynum cannabinum
Arabis hirsuta
Aralia spinosa
Arisaema triphyllum
Aruncus sylvester
Asclepias syriaca
Asclepiodora viridis
Asimina triloba
Asparagus officinalis
Aspidium marginale
Belamcanda chinensis
Benzoin aestivale
Blephilia ciliata
Botrychium virginianum
Brassica nigra
Buchloë dactyloides
Camptosorus rhizophyllus
Capsella bursa-pastoris
Cardamine bulbosa
C. pennsylvanica
Carduus crispus
Carex stricta
Castalia tuberosa
Castilleja coccinea
Caulophyllum thalictroides
Ceratophyllum demersum
Chaerophyllum procumbens
Chara foetida
Chenopodium album
Chimaphila maculata
Chrysanthemum leucanthemum
Cibotium schiedei
Cichorium intybus
Cicuta bulbifera
C. maculata
Cimicifuga racemosa
Cirsium arvense
Cladonia rangiferina
Conocephalum conicum
Crepis biennis
Crocus vernus
Cunila origanoides
Cuscuta sp.
Cynoglossum virginianum
Cyperus papyrus
Cystopteris fragilis
Dactylis glomerata
Daucus carota
Decodon verticillatus
Dentaria laciniata
Dianthera americana
Dicentra canadensis
Diospyros virginiana
Dipsacus sylvestris
Dodecatheon meadia
Eleusine indica
Epipactis tesselata
Equisetum arvense
Eragrostis cilianensis
Erigeron philadelphicus
Eupatorium purpureum
Euphorbia corollata
Evonymus obovatus
Galinsoga parviflora
Galium aparine
Gaultheria procumbens
Geranium maculatum
Geum vernum
Ginkgo biloba
Gillenia stipulata
Hedera helix
Heracleum lanatum
Heuchera sanguinea
H. villosa
Hibiscus moscheutos
Hieracium venosum
Houstonia caerulea
H. longifolia
Hydrophyllum appendiculatum
Hypericum perforatum
Ilex opaca
Impatiens pallida
Iodanthus pinnatifidus
Iris germanica
Juniperus horizontalis
Krigia amplexicaulis
Lactuca canadensis
Lamium purpureum
Laportea canadensis
Leonurus cardiaca
Lepachys pinnata
Lepidium campestre
L. virginicum
Liriodendron tulipifera
Lithospermum arvense
L. canescens
Lychnis alba
Lycopodium lucidulum
Lygodium japonicum
Lysimachia nummularia
L. quadrifolia
Malva rotundifolia
Medicago lupulina
Melilotus alba
Menyanthes trifoliata
Nepeta hederacea
Nephrolepis cordifolia
N. exaltata *var.* bostoniensis
Oenothera fruticosa
Onoclea sensibilis
Osmorhiza claytoni
O. longistylis
Osmunda regalis
Oxalis stricta
Paeonia officinalis
Panax quinquefolium
Pastinaca sativa
Pedicularis canadensis
Pentstemon laevigatus
Phacelia purshii
Phlox divaricata
Physocarpus opulifolius
Picea abies
Pinus strobus
Plantago lanceolata
P. major
Platanus occidentalis
Poa pratensis
Podophyllum peltatum
Polemonium reptans
Polygonatum biflorum
Polygonum amphibium
Polypodium aureum
P. longifolium
P. polypodioides
P. vulgare
Polystichum acrostichoides
P. lonchitis
Potamogeton crispus
P. natans
P. pectinatus
Potentilla canadensis
P. recta
Prunella vulgaris
Prunus persica
Psilotum nudum
Psoralea pedunculata
Pteris aquilina
Ranunculus septentrionalis
Rhus canadensis
Ruellia ciliosa
Rumex acetosella
R. crispus
Sagittaria latifolia
Salvia lyrata
Salvinia natans
Sambucus canadensis
Saponaria officinalis
Sarracenia purpurea
Sedum stoloniferum

Selaginella braunii
S. kraussiana
Senecio aureus
Sida hermaphrodita
Silene virginica
Silphium perfoliatum
S. terebinthinaceum
Smilacina racemosa
S. stellata
Solanum carolinense
S. dulcamara
Spartina michauxiana
Specularia perfoliata
Stellaria media
Symphoricarpos orbiculatus
Symplocarpus foetidus
Taraxacum officinale
Thalictrum dioicum
Tillandsia usneoides
Tragopogon pratensis
Trifolium hybridum
T. pratense
T. repens
Trillium sessile
Typha angustifolia
Umbilicaria delineata
Uvularia perfoliata
Valeriana pauciflora
Valerianella radiata
Verbena angustifolia
Veronica arvensis
V. officinalis
Viburnum opulus
Vicia cracca
Viola papilionacea
Vitis labrusca
Vinca minor
Yucca filamentosa
Zebrina pendula
Zizia aurea

The inhibitory activity of plant tissues varied with (*a*) species of bacteria tested, (*b*) part of plant used, (*c*) stage in which plant was collected, and (*d*) temperature under which plant was growing at time of collection. Other variations in inhibitory activity may be due to (*e*) method of extraction, (*f*) culture medium used, and (*g*) diffusibility of the extract through agar.

The antibacterial substances found in *Asarum canadense*, *Sanguinaria canadensis*, and *Taxus canadensis* were found to be thermostable. The antibacterial substance in *S. canadensis* was not lost upon drying.

Summary

Water extracts of 231 species of higher plants were tested for antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, *Erwinia carotovora*, and *Phytomonas tumefaciens*. Eighteen of the species showed sufficiently high antibacterial effects against one or more of the organisms to warrant their inclusion in table 1.

The author wishes to express appreciation to Dr. B. S. MEYER for his invaluable assistance given throughout the progress of the study and preparation of the paper and to acknowledge the aid in collecting and classifying plant specimens given by Mr. and Mrs. A. E. HYDER and Mr. CONRAD ROTH of Portsmouth, Ohio, and BETTY GREENE and Dr. J. N. WOLFE of Ohio State University. Pure cultures of the test organisms were donated by Dr. G. L. STAHLY and Dr. W. G. STOVER of Ohio State University.

CISNE, ILLINOIS

LITERATURE CITED

1. ABRAHAM, E. P.; CHAIN, E.; FLETCHER, C. M.; FLOREY, H. W.; GARDNER, A. D.; HEATLEY, N. G.; and JENNINGS, M. A. Further observations on penicillin. Lancet 2:177–189. 1945.
2. CAVALLITO, C. J.; BAILEY, J. H.; and BUCK, J. S. Antibacterial principle of *Allium sativum*. III. Its precursor and "essential oil of garlic." Jour. Amer. Chem. Soc. 67:1032–1033. 1945.
3. CAVALLITO, C. J.; BAILEY, J. H.; and KIRCHNER, F. K. Antibacterial principle of *Arctium minus*. I. Isolation, physical properties, and antibacterial action. Jour. Amer. Chem. Soc. 67: 948–950. 1945.
4. CHEN, S. L.; CHENG, B. L.; CHEN, W. K.; and TANG, P. S. An antibiotic substance in the Chinese water chestnut, *Eleocharis tuberosa*. Nature (London) 156:234. 1945.
5. GRAY, ASA. Gray's new manual of botany. 7th ed. Revised by B. L. ROBINSON and M. L. FERNALD. American Book Co., New York. 1908.

6. HEATLEY, N. G. An antibiotic from *Crepis taraxacifolia*. Brit. Jour. Expt. Path. **25**:208–211. 1944.
7. LUCAS, E. H., and LEWIS, R. W. Antibacterial substances in organs of higher plants. Science **100**:597–599. 1944.
8. OSBORN, E. M. On the occurrence of antibacterial substances in green plants. Brit. Jour. Expt. Path. **24**:227. 1943.
9. PRATT, R.; DANIELS, T. C.; EILER, J. J.; GUNNISON, J. B.; KUMLER, W. D.; ONETO, J. F.; STRAIT, L. A.; SPOEHR, J. A.; HARDIN, G. J.; MILNER, J. W.; SMITH, J. H. C.; and STRAIN, H. H. Chlorellin, an antibacterial substance from *Chlorella*. Science **99**:351–352. 1944.
10. TOKIN, B. Effect of phytoncides upon protozoa. Amer. Rev. Sov. Med. **1**:237–239. 1944.
11. TOROPTSEV, I. V., and FILATOVA, A. G. The use of phytoncides in treatment of infected wounds. Amer. Rev. Sov. Med. **1**:244–250. 1944.
12. SANDERS, DOROTHY W.; WEATHERWAX, P.; and MCCLUNG, L. S. Antibacterial substances from plants collected in Indiana. Jour. Bact. **49**:611–615. 1945.
13. SEEGAL, BEATRICE C., and HOLDEN, MARGARET. The antibiotic activity of extracts of Ranunculaceae. Science **101**:413–414. 1945.

SELECTIVE HERBICIDAL EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED TO TURF IN DRY MIXTURES WITH FERTILIZER

PAUL C. MARTH[1] AND JOHN W. MITCHELL[2]

Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is effective as a herbicide when applied as a dust to the foliage of various kinds of weeds (**2**) or to soil in which these plants are growing (**1, 6, 7, 8**). In general, most lawn grasses, such as bluegrass (*Poa pratensis*), red top (*Agrostis alba*), and red fescue (*Festuca rubra*), have proved to be relatively resistant to 2,4-D but have shown, in varying degrees, a temporary checking in their rate of growth following use of the sprays (**5**). Certain grasses, such as the bents, have proved to be much more sensitive and have exhibited marked injury with applications that ordinarily have but a slight effect on bluegrass. To speed up recovery of grasses from this initial growth-depressing effect of the herbicide, it was found helpful to supply nutrient material, such as urea, to them at the time of treatment (**3**). Spray application of a water mixture of urea (supplying 60 lb. per acre) and 2,4-D (1.5 lb. per acre) had beneficial effects on recovery of bluegrass. Preliminary experiments with dry application of complete fertilizers containing various amounts of 2,4-D have also given similar effects (**4**). The present experiments were undertaken to determine (*a*) the amounts of 2,4-D applied dry in mixture with complete fertilizer that would be required per acre selectively to eradicate weeds from turf and (*b*) the effect of such mixtures on the growth of turf from applications at various seasons of the year.

[1] Physiologist, [2] Senior Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Md.

Methods

2,4-Dichlorophenoxyacetic acid was used throughout these experiments, except where the sodium, ammonium, or morpholine salts of the acid were substituted for comparative purposes. The mineral fertilizer was of commercial grade, 10–6–4 (N, P_2O_5, K_2O) analysis, purchased on the open market, and com-

monly used for fertilizing lawns and gardens.

A mechanically operated barrel-type concrete mixer was used in preparing the fertilizer plus 2,4-D mixtures. The desired weighed amount of 2,4-D was first mixed thoroughly by hand with 1 lb. of fertilizer prior to adding this mixture to the desired weighed amount of fertilizer (usually about 20 lb.) as it revolved in the mixer, and the mixing operation was then continued for a period of 10 minutes.

All fertilizer treatments were at the rate of 600 lb. per acre, the amount generally recommended for stimulating the growth and development of sod. All mixtures were applied on the same or succeeding day after mixing.

The area of established sod selected for experimentation was composed mainly of Kentucky bluegrass, red top, and fescue grasses heavily and uniformly infested with narrow-leaved plantain (*Plantago lanceolata*). Other weedy species present included sheep sorrel (*Rumex acetosella*), black medic (*Medicago lupulina*), and white clover (*Trifolium repens*). Two sets of plots, thirty each, were marked off; treatments on one set were applied on November 6, 1945; on the other, on March 22, 1946. Individual plots were 2 feet wide and 25 feet long, and a 1-foot border was left between plots. At each date of treatment the thirty plots were divided into five replicates, and the six different treatments were assigned at random to plots within blocks. The applications consisted of 0, 1.5, 3, 6, and 9 lb. per acre of 2,4-D mixed with fertilizer and of an unfertilized control that received no 2,4-D. The weighed amount of fertilizer mixture was applied as carefully and evenly as possible to each plot by hand when the foliage was free of surface moisture.

In another experiment on larger areas ($\frac{1}{6}$–1 acre) a fertilizer attachment on a 12-foot lawn drill drawn by a tractor was used. Sufficient 2,4-D (acid) was mixed with fertilizer by hand-shoveling to deliver 4 lb. of 2,4-D in 300 lb. of fertilizer per acre; this was applied on March 15, 1946, to a rectangular lawn area approximately 1 acre in size. Again on March 26 the same distributor was used in treating three adjacent $\frac{1}{6}$-acre lawn areas with 4 lb. per acre of the morpholine, sodium, and ammonium salts of 2,4-D, respectively. Since the area had already been fertilized with 300 lb. per acre of 10–6–4 fertilizer, the various salts were applied in mixture with fine sand.

Clippings were obtained from all plots by use of a small power mower adjusted to cut at a height of 1.5 inches and fitted with a grass-catcher. Fresh weights of both grass and weed foliage were obtained after the clippings had been hand-sorted.

Results

LATE FALL APPLICATION.—On April 4, 1946, there was no significant difference in the growth of grass between the fertilized control plots (no 2,4-D) and others that received varying amounts of 2,4-D with fertilizer on the preceding November 6 (table 1). In comparison with the fertilized control plot, however, the addition of both 6 and 9 lb. per acre of 2,4-D caused a marked intensification of green color in the grass foliage so that these plots, by visual inspection, could very readily be selected from among the various treatments. 2,4-D applications of 1.5 and 3 lb. per acre also caused greening of the grass but to a lesser degree. This difference in coloration persisted until about June 1.

From the standpoint of weed eradica-

tion even the smallest amount of 2,4-D (1.5 lb. per acre) applied in the dry form together with fertilizer gave approximately 100% control of narrow-leaved plantain. At the 6- and 9-lb. rates the small amount of weed clippings obtained consisted almost entirely of sheep sorrel, a plant with underground stolons that has sometimes been found difficult to control by means of the usual 2,4-D spray treatment.

TABLE 1

EFFECT OF FALL APPLICATIONS OF 2,4,-D IN DRY MIXTURE WITH COMMERCIAL (10–6–4) FERTILIZER ON GROWTH OF GRASS AND DESTRUCTION OF WEEDS IN PLOTS OF 50 SQUARE FEET TREATED NOVEMBER 6, 1945. CLIPPINGS OBTAINED APRIL 4, 1946

TREATMENT		FRESH CLIPPING WEIGHTS (AVERAGE OF 5 PLOTS)		PERCENTAGE OF WEEDS
2,4-D (lb./acre)	10–6–4 fertilizer (lb./acre)	(Grass) (gm.)	Weeds (gm.)	
0.....	0	145.4*	28.6	16.9
0.....	600	329.0	62.2	16.5
1.5.....	600	332.6	7.4	2.5
3.0.....	600	343.0	6.0	1.4
6.0.....	600	338.0	2.4	0.7
9.0.....	600	337.4	1.8	0.5

* Differences of 78.6 gm. are significant at the 5% level.

EFFECT ON GRASS SEED PRODUCTION.—After the grass had developed following clipping on April 4, differences in the number of seed stalks of grass on the different plots became apparent. The seed heads, which consisted almost entirely of bluegrass, were permitted to develop fully, and on June 6, 1946, the bluegrass seed heads and straw were harvested by individual plots. At this time the straw had just begun to wither, and the seeds were judged to have reached maturity. After drying for 2 weeks in a greenhouse at approximately 70°–100° F., the seed heads were further dried overnight in a ventilated oven at 100° F.; the seeds were then rubbed out by hand and weighed.

Although the results on seed production are not sufficient for final conclusions, it is of interest that the average seed (plus chaff) production per plot amounted to 4.2, 17.0, 23.5, 22.1, 17.5, and 22.5 gm., respectively, for the control, fertilized control, and fertilizer plus 1.5, 3, 6, or 9 lb. of 2,4-D per acre. From the standpoint of grass seed production the significance of these results lies principally in the fact that the plots that received 2,4-D treatment were free of weeds and therefore relatively free of weed seeds. Further experiments are in progress to determine the possible effects of comparable applications of 2,4-D to soil on the yield and viability of grass seeds.

SPRING APPLICATION.—Marked differences in the growth of grass were obtained from dry applications on March 22 of fertilizer plus 2,4-D mixtures (table 2). On both the May 6 and the July 9 clipping dates plots that had received 1.5 lb. of 2,4-D per acre together with fertilizer produced a significantly greater amount of grass clippings than did plots that received fertilizer alone. When 2,4-D was applied at 3 and 6 lb. per acre, the weights of grass clippings were slightly greater than in the controls (fertilized). At the first clipping these latter differences were not statistically significant, but at the later clipping (July 9) they were found to be significant. Larger amounts of 2,4-D were injurious to the growth of grass (table 2). The reduction of 182.2 gm. in average clipping weight of grass on May 6 caused by 9 lb. of 2,4-D per acre in comparison with the fertilized control was found to be highly significant. By July 9, however, the grass

had recovered so that there was no significant difference between the weights of clippings from the fertilized control and from plots treated with 9 lb. of 2,4-D per acre. The injurious effects of 2,4-D applied at the rate of 9 lb. per acre were clearly evident in the appearance of the grass, the blades being narrower and shorter, although deeper green in color.

As with fall application, an intensification of green coloration of the grass foliage was noted at the 3-, 6-, and 9-lb. rates of 2,4-D applications in spring, in comparison with the fertilized control plots.

The weed-killing effects of 2,4-D were equally effective in the fall and spring applications under the condition of these experiments (fig. 1).

TREATMENT OF LARGE LAWN AREAS.—The feasibility of applying dry 2,4-D mixtures on a larger scale for selective herbicidal effects was tested during the spring of 1946. All the treatments, with

TABLE 2

EFFECT OF SPRING APPLICATION OF 2,4-D IN DRY MIXTURE WITH COMMERCIAL (10-6-4) FERTILIZER ON SUBSEQUENT YIELD OF GRASS AND WEED CLIPPINGS IN PLOTS OF 50 SQUARE FEET TREATED MARCH 22, 1946

TREATMENT		FRESH CLIPPING WEIGHTS (AVERAGE OF 5 PLOTS)					
		May 6, 1946			July 9, 1946		
2,4-D (lb./acre)	10-6-4 fertilizer (lb./acre)	Grass (gm.)	Weeds (gm.)	Percentage of weeds	Grass (gm.)	Weeds (gm.)	Percentage of weeds
0.....	0	366.4*	53.6	11.4	304.4†	92.5	23.2
0.....	600	749.6	182.4	21.5	384.8	166.8	30.1
1.5.....	600	931.8	12.2	1.1	530.8	24.2	4.2
3.0.....	600	805.8	6.2	0.7	485.2	10.6	2.0
6.0.....	600	756.2	1.8	0.2	427.2	8.9	2.0
9.0.....	600	560.6	1.4	0.2	380.8	9.4	2.4

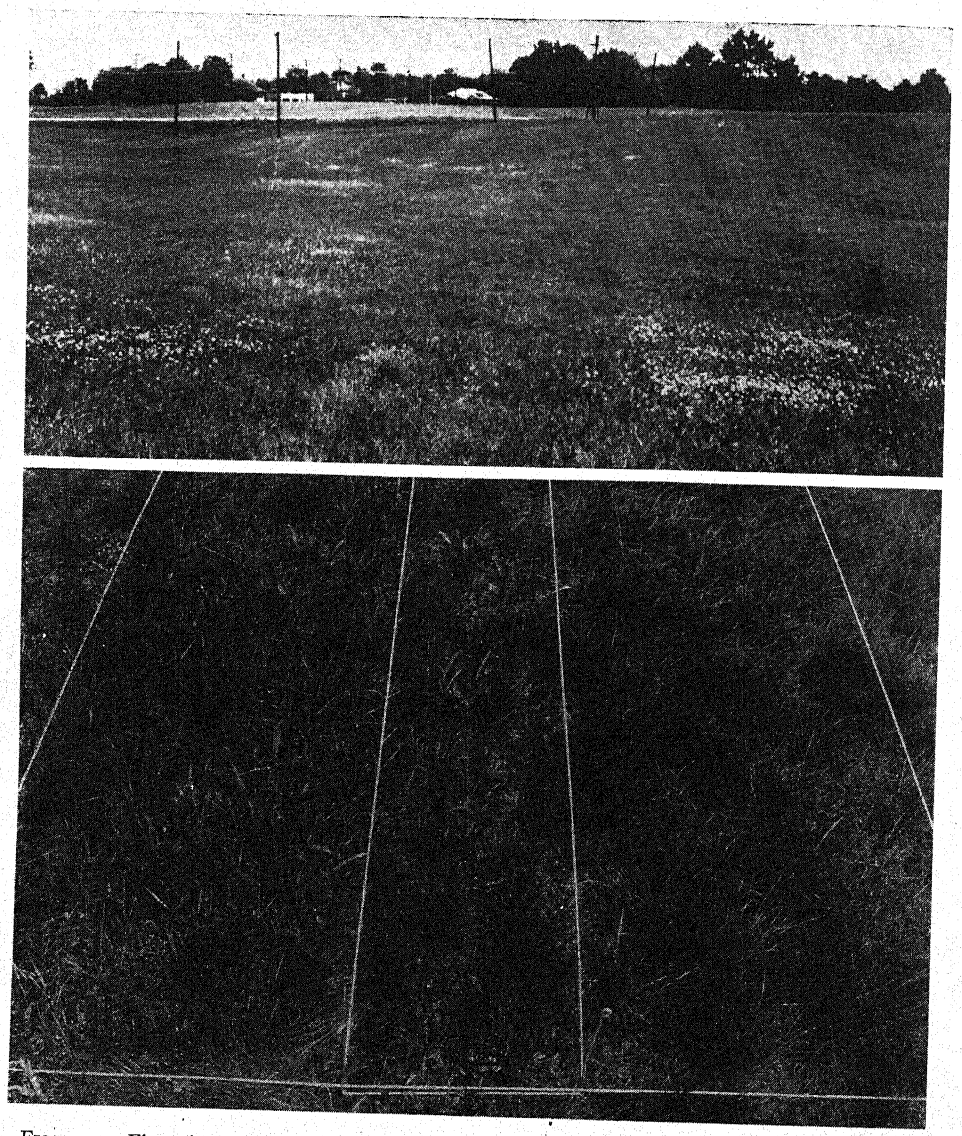
* Differences of 146.0 gm. are significant at the 5% level.

† Differences of 69.3 gm. are significant at the 5% level.

TABLE 3

HERBICIDAL EFFECTS OF VARIOUS SALTS AND THE ACID FORM OF 2,4-D APPLIED AS DRY MIXTURES AT RATE OF 4 LB. PER ACRE TO GRASS SOD

TREATMENT	PERCENTAGE OF CLIPPINGS THAT CONSISTED OF WEED FOLIAGE					
	Sample no.					Average
	1	2	3	4	5	
Control.....	60.0	54.4	25.2	56.4	46.0	48.4
2,4-D acid.....	10.2	13.6	12.8	14.0	10.4	12.2
2,4-D ammonium salt.....	1.0	1.6	1.2	4.0	1.6	1.9
2,4-D sodium salt.....	14.4	11.2	10.8	16.0	12.4	13.0
2,4-D morpholine salt.....	12.4	16.0	22.0	20.0	12.0	16.5



FIGS. 1–2.—Fig. 1 (*top*), reduction in stand of Dutch White clover in Kentucky bluegrass sod following treatment on March 15, 1946, with ammonium salt of 2,4-D at rate of 4 lb. per acre applied dry in mixture with sand. Area ($\frac{1}{6}$ acre) at center of photograph was treated; adjacent areas left untreated. Photographed July 9, 1946. Fig. 2 (*bottom*), eradication of narrow-leaved plantain by application on March 22, 1946, of 2,4-D applied dry in mixture with fertilizer. *Left*, plot which received fertilizer only; *center*, untreated guard strip; *right*, plot which received fertilizer containing 2,4-D applied at rate of 3 lb. per acre. In each instance complete fertilizer (10–6–4) was applied at rate of 600 lb. per acre. Photographed May 15, 1946.

either the acid or the salt forms of 2,4-D, caused the sod to develop a dark-green coloration as compared with that of adjacent areas which received fertilizer only (fig. 1). This deep-green color persisted until fall. On May 29, clippings were obtained on strips 18 inches wide and 25 feet long selected at random at five different locations in the treated areas (table 3).

The high percentage of weed clippings found in the control areas consisted mainly of narrow-leaved plantain, sheep sorrel, and white clover. The ammonium salt reduced the amount of weeds more effectively than did the other salts. Direct comparison between the salts and the acid cannot be made because of the difference in time and manner of application (the salts were applied in mixture with sand, the particles of which were more uniform in size than were those of the fertilizer). The acid and the sodium salt treatments were about equal in effectiveness, while the morpholine salt was relatively ineffective as a herbicide when applied in this manner.

Discussion

The use of a mixture of 2,4-D and complete fertilizer for weed control and fertilizing purposes would appear to have a number of advantages over other methods of treating grassy areas with 2,4-D. Except for the added cost of incorporating 2,4-D in the mixture, the treatment would cause no additional expense in ridding such areas of weeds. The herbicidal treatment would fit into the regular schedule of spring or fall fertilization recommended for lawn care, and the use of costly labor and equipment for applying a special 2,4-D spray treatment for weed-killing could therefore be avoided.

General recommendations for the use of fertilizer plus 2,4-D mixture cannot be made on the basis of the present limited experiments. Results of the present tests indicate, however, that the inclusion of a small amount of 2,4-D in lawn fertilizer mixtures would prove effective in destroying a large percentage of established weeds and would discourage reinfestation from weed seed present in the soil.

It has been shown in previous experiments (1, 4, 6), as well as in those reported herein, that many weedy plants are quite sensitive to 2,4-D absorbed through the roots. The effects of the chemical appear to be translocated rapidly throughout the entire plant within a short time after it is absorbed. This method of applying 2,4-D to the soil surface appears to offer promise in eradicating certain weeds that have large underground storage organs which frequently develop new shoots and reestablish the plant following spray treatment of the tops.

These experiments were conducted with freshly prepared mixtures of 2,4-D and fertilizer. Further experiments are in progress on the possible effect of the type of fertilizer used and its method of preparation and storage on the stability of 2,4-D contained in such mixtures.

Summary

1. Dry mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D) and complete 10-6-4 fertilizer were applied to established weedy turf on November 6, 1945, and March 22, 1946, on plots 2 feet wide and 25 feet long. The fertilizer was used at the uniform rate of 600 lb. per acre and contained varying amounts of 2,4-D, namely, 0, 1.5, 3, 6, and 9 lb. per acre. The effects of the various treatments on the growth of turf and on the eradication of weeds were measured by sorting and weighing separately the clippings of weeds and of grass from the individual plots.

2. No significant difference, in comparison with the fertilized control, was found in the weight of grass clippings on April 4, 1946, from any 2,4-D fertilizer treatments that were applied on November 6, 1945. Such weeds as narrow-leaved plantain, black medic, and bur clover were eradicated almost 100% by the lowest amount (1.5 lb. per acre) of 2,4-D used in the fertilizer mixture. At the highest rates (6 and 9 lb. per acre) the green coloration of the grass was visibly darker than in the fertilized control plots until about June 1.

3. Application on March 22, 1946, of both 6 and 9 lb. per acre of 2,4-D in mixture with fertilizer caused visible injury to the turf so that the weight of grass clippings on May 6, 1946, was significantly lower than in the fertilized controls. However, in these experiments mixtures containing 1.5 and 3 lb. per acre of 2,4-D with fertilizer induced a highly significant increase in growth of the turf. Weed eradication was about equal to that resulting from treatment on November 6.

4. The bluegrass in all plots treated on November 6, 1945, was allowed to develop and to mature seed stalks, which were collected on June 6, 1946. The average dry weight of seed and chaff was 4.2, 17.0, 23.5, 22.1, 17.5, and 22.5 gm., respectively, for the control, fertilized control, and fertilizer plus 1.5, 3, 6, or 9 lb. of 2,4-D per acre. Owing to the absence of weeds, grass seed from the plots treated with 2,4-D was free of weed seeds.

5. Applications of the ammonium, sodium, and morpholine salts of 2,4-D mixed in dry sand were made on March 15, 1946, at the rate of 4 lb. per acre of each salt to individual $\frac{1}{6}$-acre plots of weedy turf. Samples of both weed and grass foliage from these plots on May 29, 1946, indicate that these salts of 2,4-D are effective in eradicating turf weeds when applied in dry form. The ammonium salt proved to be superior to the other salts in its herbicidal effects.

6. Although the present experiments are not sufficient to justify recommendations regarding the use of 2,4-D–fertilizer mixtures on turf, the lowest rate (1.5 lb. per acre) of 2,4-D that was used in combination with fertilizer caused almost 100% weed eradication. The chemical appears to be very effective when applied broadcast. It seems to be absorbed from the soil by the roots of sensitive weedy plants, and, once it has been taken up, the 2,4-D stimulus appears to be translocated rapidly throughout the entire plant.

BUREAU OF PLANT INDUSTRY,
SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

LITERATURE CITED

1. HAMNER, C. L.; MOULTON, J. E.; and TUKEY, H. B. Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid on germination and development of seedlings. BOT. GAZ. 107: 352–361. 1946.
2. MARTH, P. C.; DAVIS, F. F.; and MITCHELL, J. W. Herbicidal properties of 2,4-dichlorophenoxyacetic acid applied in dusts containing hygroscopic agents. BOT. GAZ. 107:129–136. 1945.
3. MARTH, P. C., and MITCHELL, J. W. Effect of spray mixtures containing 2,4-dichlorophenoxyacetic acid, urea, and Fermate on the growth of grass. BOT. GAZ. 107:417–424. 1946.
4. ———, and ———. Period of effective weed control by the use of 2,4-dichlorophenoxyacetic acid. Sci. 104:77–79. 1946.
5. MITCHELL, J. W., and MARTH, P. C. Effects of 2,4-dichlorophenoxyacetic acid on the growth of grass plants. BOT. GAZ. 107:276–284. 1945.
6. ———, and ———. Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 107:408–416. 1946.
7. NUTMAN, P. S.; THORNTON, H. G.; and QUASTEL, J. H. Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant growth substances. Nature 155:498–500. 1945.
8. SLADE, R. E.; TEMPLEMAN, W. G.; and SEXTON, W. A. Plant growth substances as selective weed-killers. Nature 155:497–498. 1945.

RESPONSE OF POTATO PLANTS TO SPRAY APPLICATIONS OF CERTAIN GROWTH-REGULATING SUBSTANCES[1]

ORA SMITH, M. A. BAEZA, AND J. H. ELLISON

Introduction

For years farmers have had heavy losses from potatoes sprouting excessively and shriveling in storages which could not be kept at temperatures of 40° F. or lower. Potato-chip manufacturers are constantly troubled with the production of dark-colored chips. This darkening is associated with high reducing-sugar content which develops at low storage temperatures. Higher storage temperatures would prevent accumulation of reducing sugars but would result in excessive sprout growth of tubers. Potatoes can now be treated in commercial quantities in storage with the methyl ester of α-naphthaleneacetic acid in liquid form (8) which prevents or greatly retards sprout growth at relatively high temperatures. The problem would be further simplified if some substance sprayed on the plants in the field would similarly retard growth of sprouts in storage and not reduce yields or quality of tubers. It was the purpose of this investigation to determine the effects of several compounds sprayed on potato plants on the mineral nutrient content of petioles, specific gravity and reducing-sugar content of tubers, yields of tubers, and effects on sprout retardation in subsequent storage.

Material and methods

Field tests were conducted on Lordstown silt loam soil of pH 5.0, using the Sebago variety, planted May 15, 1945, in rows 34 inches apart and with seed pieces at 11-inch intervals. All plots were fertilized alike at the time of planting with 2400 lb. to the acre of 5–10–10 fertilizer applied in equal depth bands through the planter.

The experiment included control plots and plots treated with four different growth-regulators applied in sprays: (*a*) sodium 2,4-dichlorophenoxyacetate at 10 p.p.m.; (*b*) methyl ester of α-naphthaleneacetic acid (NA) at 10,000 p.p.m. first application, 4000 p.p.m. thereafter; (*c*) indoleacetic acid (IA) at 150 p.p.m.; and (*d*) α-naphthaleneacetic acid (NA) at 150 p.p.m.

The spray solutions were made up in the following manner: The powder form of 70% sodium 2,4-dichlorophenoxyacetate directly soluble in water was used. Two parts of the methyl ester of NA plus 1 part of polyethylene glycol 400 were diluted with water to the desired strength and kept agitated during spraying. The concentration of the methyl ester of NA for the first application (10,000 p.p.m.) was so strong that marked injury to leaves resulted; hence, for all subsequent applications concentrations were reduced to 4000 p.p.m. Indoleacetic acid (IA) and α-naphthaleneacetic acid (NA) were each dissolved in 5 ml. of 95% ethyl alcohol and then diluted with water.

There were four dates of application: July 14 and 28 and August 11 and 25. One series of plots received applications on July 14 only, one series on July 14 and 28, one series on July 14 and 28 and August 11, and one series on all four dates. The sprays were applied through a knapsack sprayer at the following rates per acre: July 14, 55 gallons; July

[1] Paper no. 283, Department of Vegetable Crops, Cornell University, Ithaca, N.Y.

28, 82 gallons; August 11 and 28, 110 gallons. Plots were of single rows, 25 feet long with guard row between treatments and 5-foot alleys between replication blocks. All treatments were in quadruplicate.

Rapid chemical tests of petiole tissue were made on August 22 and September 8, using the methods of PEECH and ENGLISH (6). Plots were harvested and yield data were obtained on October 22, 1945. The specific gravity of the total tubers from each plot was obtained by differences in weight in air and in water. Samples from each plot were placed in storage at 40° F. and at 50° F. on November 15, 1945.

On February 1, 1946, the reducing-sugar content of tubers held at 40° F. was determined by a modified SHAFFER-SOMOGYI method (7).

On February 13, 1946, the number and weight of sprouts of the lots stored at 50° F. were obtained.

Results

GROWTH IN THE FIELD.—Two days after the first application plants of all plots sprayed with the methyl ester of NA showed severe upward rolling of the leaves, with some of the leaves severely injured. It appeared, however, that only those leaves receiving direct application of the chemical were injured.

SMITH *et al.* (9) found that the methyl ester of NA applied to the soil as a preemergence spray is an effective selective herbicide in potato fields. Although the 10,000 p.p.m. applications in the present experiment did not act as a complete herbicide to the potato plants, it was obvious that this was too high a concentration when the methyl ester of NA was sprayed on the plants; therefore, at the three subsequent applications concentrations were reduced to 4000 p.p.m. Two weeks after the first application plants treated with this substance had few flowers, while the untreated were flowering profusely. Most of the treated plants had somewhat recovered from the injury. Two weeks after the last application it was impossible to differentiate among those that had received one and those which had received more than one application. No effects were noticeable in the field on plants sprayed with the other growth substances.

RAPID CHEMICAL TESTS OF TISSUES.—Analyses of petiole samples taken between the third and fourth spray applications showed that there were no significant differences in nitrate nitrogen, calcium, or magnesium content of petioles owing to treatments or number of applications (table 1). The phosphorus content of petioles from plants sprayed with the methyl ester of NA was significantly higher than in all other treatments, and the potassium content of petioles from all spray treatments was significantly higher than in the untreated plants.

Results of analyses of petioles 2 weeks after the final applications had been made are shown in table 2. Petioles from plants of all treatments, except those sprayed with NA, were significantly higher in nitrate nitrogen than the untreated ones. At this time plants treated with IA and with the methyl ester of NA were significantly higher in phosphorus and potassium than the untreated ones. No significant differences among treatments were found in content of calcium or magnesium.

EFFECTS ON YIELDS.—Treatment with the methyl ester of NA significantly reduced the total yield and yield of U.S. no. 1 size potatoes (table 3). None of the other growth substances influenced yield significantly, and the number of spray

TABLE 1

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON CONTENT OF NUTRIENTS IN PETIOLES. AUGUST 22, 1945

Treatment	Number of applications																			
	Nitrate nitrogen (p.p.m.)				Phosphorus (p.p.m.)				Potassium (p.p.m.)				Calcium (p.p.m.)				Magnesium (p.p.m.)			
	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
								***				***								
Sodium 2,4-dichlorophenoxyacetate	800	608	880	763	45	45	55	48	8320	5230	7040	6880	600	600	500	580	35	25	25	28
Methyl ester of naphthaleneacetic acid	800	960	880	886	75	80	80	78	7200	10400	8000	8533	500	600	700	600	25	25	30	27
Indoleacetic acid	800	768	320	629	60	60	45	55	9600	6400	6400	7466	500	600	600	580	30	25	40	32
Naphthaleneacetic acid	640	560	360	587	50	45	50	48	6720	5600	7520	6613	600	600	700	590	25	25	30	27
Untreated*	688	688	688	688	50	50	50	50	4800	4800	4800	4800	600	600	600	600	30	30	30	30
Av.	746	717	666	.....	56	56	56	....	7328	6496	6752	.....	560	600	620	.....	29	26	31	....
	Not significant				L.S.D.—Treatment at .05 = 12 at .01 = 17 *** at .001 = 24				L.S.D.—Treatment at .05 = 1196 at .01 = 1661 ***at .001 = 2310				Not significant				Not significant			

* Samples from only two untreated plots taken; each figure represents average of same two plots.

TABLE 2

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON CONTENT OF NUTRIENTS IN PETIOLES. SEPTEMBER 8, 1945

TREATMENT	NUMBER OF APPLICATIONS																								
	Nitrate nitrogen (p.p.m.)					Phosphorus (p.p.m.)					Potassium (p.p.m.)					Calcium (p.p.m.)					Magnesium (p.p.m.)				
	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.
					**					**					**										
Sodium 2,4-dichlorophenoxyacetate	450	300	600	450	450	30	50	65	100	61	7500	4950	7500	6000	6488	800	1000	800	800	850	7.5	17.5	7.5	7.5	10.0
Methyl ester of naphthaleneacetic acid	750	825	825	375	694	90	70	90	95	86	7500	8250	8250	6750	7688	700	800	700	900	775	5.0	7.5	7.5	5.0	6.2
Indoleacetic acid	450	600	300	600	488	75	80	70	95	80	7500	8250	8250	7500	7850	700	800	800	800	775	12.5	10.0	12.5	12.5	10.2
Naphthaleneacetic acid	300	300	300	525	356	70	65	80	95	78	6750	6000	5700	5400	5962	800	800	900	900	850	10.0	12.5	25.0	22.5	17.5
Untreated*	300	300	300	300	300	65	65	65	65	65	6750	6750	6750	6750	6750	800	800	800	800	800	7.5	7.5	7.5	7.5	7.5
Av.	450	465	465	450	....	66	66	74	90	...	7200	6840	7290	6480	.....	760	840	800	840	....	8.5	11.0	12.0	11.0	.....
	L.S.D.—Treatment at .05=144 ** at .01=197					L.S.D.—Treatment at .05=14 ** at .01=19 L.S.D.—Number of applications at .05=12 at .01=17					L.S.D.—Treatment at .05=1058 ** at .01=1447					Not significant					Not significant				

* Samples from only two untreated plots taken; each figure represents average of same two plots.

TABLE 3

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON TOTAL YIELD AND YIELD OF U.S. NO. 1 SIZE POTATOES. SEBAGO VARIETY. 1945

Treatment	Bushels per acre									
	Total					U.S. no. 1 size				
	1 application	2 applications	3 applications	4 applications	Av.	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	308	300	324	306	309	268	259	295	262	271
Methyl ester of naphthaleneacetic acid	214	170	143	162	172***	159	108	86	94	112***
Indoleacetic acid	288	348	318	329	320	248	304	266	288	276
Naphthaleneacetic acid	332	321	326	337	328	283	277	287	296	286
Untreated	299	334	327	310	317	266	270	293	283	278
Av.	288	294	287	289	.....	245	244	245	245	.....
	L.S.D.—Treatment (total) at .05 = 29.3 bus. at .01 = 38.9 bus. *** at .001 = 50.6 bus. Number of applications—no significant difference					L.S.D.—Treatment (U.S. no. 1 size) at .05 = 28.6 bus. at .01 = 38.0 bus. *** at .001 = 49.4 bus. Number of applications—no significant difference				

TABLE 4

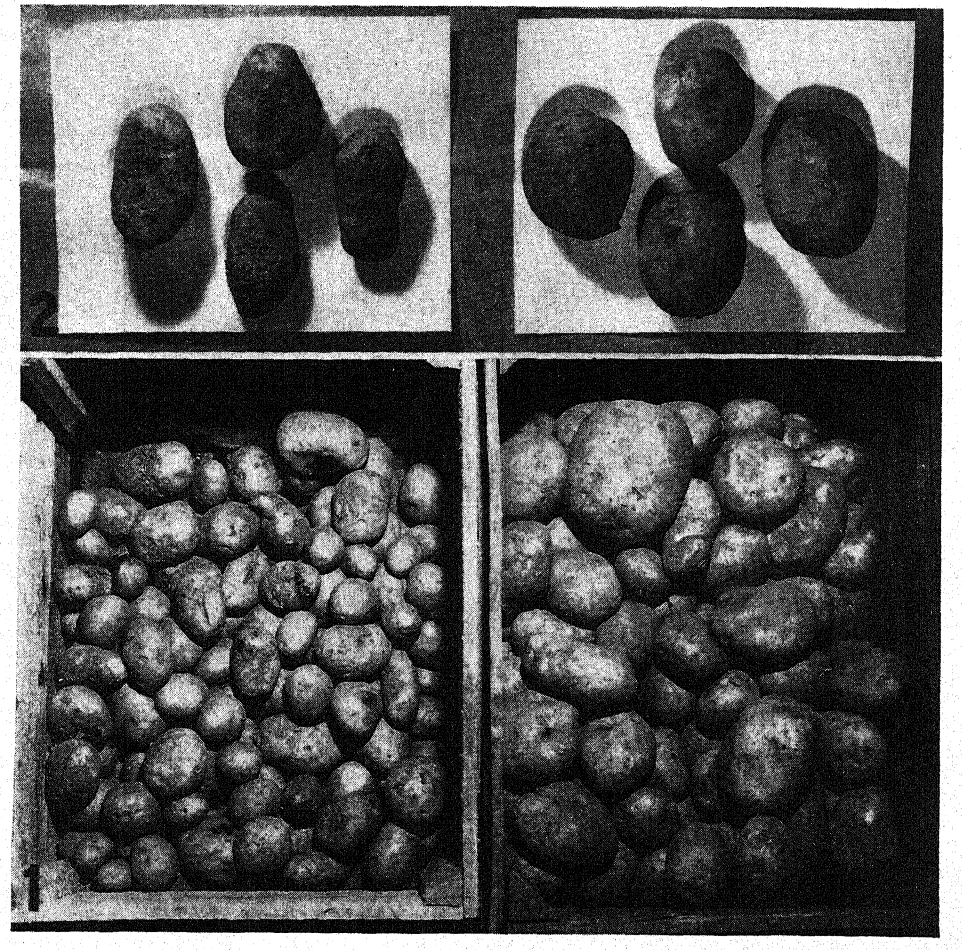
DEGREE OF INJURY EXHIBITED BY TUBERS FROM PLANTS SPRAYED WITH METHYL ESTER OF NA*

Treatment	Total number tubers	Percentage of area injured					Number with cracks
		No injury	Very little injury	25% injury	50% injury	More than 50% injury	
Untreated	138	100.0	.....	.....	.....	.....	2
Sprayed once	129	74.4	14.7	2.3	5.4	3.1	1
Sprayed twice	96	18.8	21.9	26.0	14.6	18.8	10
Sprayed three times	127	18.1	29.9	23.6	8.7	19.7	9
Sprayed four times	113	23.9	14.2	15.0	15.9	31.0	6

* Data refer to injured side of potato only.

applications had no significant effect on yield of tubers. Treatment with the methyl ester of NA significantly decreased the percentage of U.S. no. 1 size tubers as compared with the other treatments.

TUBER INJURY.—Typical injury to tubers and the size of tubers from plants which had been sprayed with the methyl ester of NA are shown in figures 1 and 2. The "pitted-scab-like" injury which was so prevalent occurred on only one side of the tuber and appeared to be confined to the underside of the tubers as they were attached to the plant. Often the opposite or upper side contained a prominent growth crack. The injury extended several millimeters into the tuber, affecting the periderm and a few cell layers of the cortex. There were marked differences in amount and severity of injury, increasing with number of spray



FIGS. 1–2.—Fig. 1, each crate contains total yield from one plot. *Left*, tubers from plants sprayed four times with methyl ester of α-naphthaleneacetic acid; note characteristic injury resembling potato scab, growth cracks, and small size of tubers. *Right*, tubers from unsprayed plants. Fig. 2, tubers on left from plants sprayed four times with methyl ester of α-naphthaleneacetic acid, tubers on right from unsprayed plants; note differences in size, shape, and surface injury.

TABLE 5

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON SPECIFIC GRAVITY OF POTATOES. SEBAGO VARIETY

TREATMENT	SPECIFIC GRAVITY				
	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	1.063	1.061	1.062	1.061	1.062
Methyl ester of naphthaleneacetic acid	1.059	1.052	1.054	1.046	1.053***
Indoleacetic acid	1.061	1.062	1.060	1.062	1.062
Naphthaleneacetic acid	1.063	1.061	1.063	1.062	1.063
Untreated	1.064	1.062	1.061	1.062	1.062
Av.	1.062**	1.060	1.060	1.059	.....

L.S.D.—Treatment
*** at .001 = .003460
L.S.D.—Number of applications
** at .01 = .0023

TABLE 6

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON REDUCING-SUGAR CONTENT DEVELOPED BY POTATOES STORED FOR 11 WEEKS AT 40° F.

TREATMENT	REDUCING SUGAR (MG./CC. JUICE)				
	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	6.16	6.77	5.45	5.68	6.01
Methyl ester of naphthaleneacetic acid	5.67	5.75	4.94	5.18	5.39
Indoleacetic acid	4.60	6.61	6.82	5.69	5.93
Naphthaleneacetic acid	6.20	6.07	4.70	6.95	5.98
Untreated	5.60	5.59	5.32	6.18	5.67
Av.	5.60	6.16	5.45	5.94	N.S.

No significant differences.

applications (table 4). A similar type of injury has been noted by ENNIS *et al.* (2) as a result of spraying potato plants with ammonium 2,4,5-trichlorophenoxyacetate.

SPECIFIC GRAVITY OF TUBERS.—A high positive correlation exists between specific gravity, starch content, and dry weight of potatoes and the texture or degree of mealiness. Hence, the mealiness of tubers can be measured by the specific gravity method, and they subsequently are available for further chemical analysis or other determinations or for planting.

Spray treatment and number of spray applications resulted in statistically significant differences in specific gravity of the tubers (table 5). Treatment with the methyl ester of NA lowered the specific gravity markedly; specific gravity in all other treatments was the same as in the untreated lots.

REDUCING-SUGAR CONTENT OF TUBERS.—After 11 weeks' storage at 40° F., reducing-sugar analyses were made of tubers from each treatment. The kind of treatment and the number of applications had no significant effect on the reducing-sugar content developed in storage (table 6).

SPROUTING OF TUBERS IN STORAGE.—At the end of 3 months' storage at 50° F. samples of twenty tubers were chosen from each lot, and data were obtained on number and weight of sprouts. Those stored at 40° F. showed no visible sprouts at that time. Table 7 shows that all spray treatments had significantly decreased the number of sprouts per tuber; very marked reduction resulted from applications of methyl ester of NA (figs. 3–4). There was no significant difference in number of sprouts per tuber as related to number of spray applications of any of the chemicals. The relationship was

TABLE 7

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON SPROUTING OF POTATOES STORED FOR 3 MONTHS AT 50° F. SEBAGO VARIETY. 1945

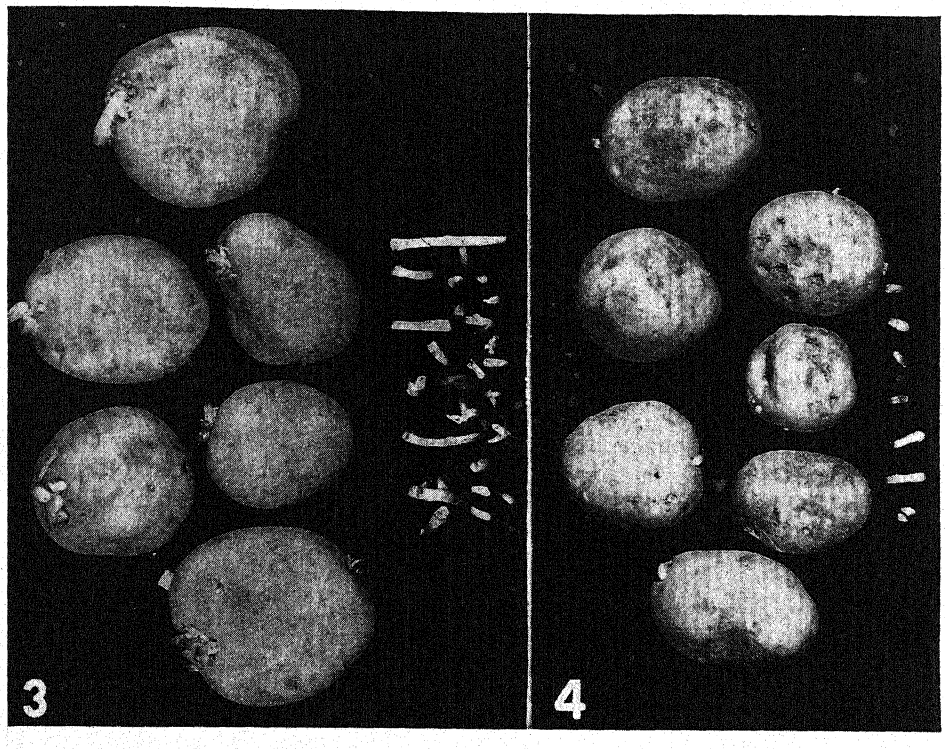
TREATMENT	NUMBER OF SPROUTS PER TUBER					WEIGHT PER SPROUT (MG.)				
	1 application	2 applications	3 applications	4 applications	Av.	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	4.5	3.9	3.8	4.6	4.2*	150	180	170	140	160**
Methyl ester of naphthaleneacetic acid	3.2	1.2	1.8	0.91	1.7***	130	120	140	160	140**
Indoleacetic acid	4.7	4.2	3.9	3.9	4.2*	200	180	190	150	180
Naphthaleneacetic acid	5.2	3.4	4.6	4.0	4.3*	160	120	190	170	160**
Untreated	5.1	6.3	5.1	4.9	5.4	220	160	150	220	190
Av.	4.5	3.8	3.8	3.4	.....	170	150	170	170	.....
	Number of applications—no significant difference L.S.D.—Treatment * at .05 = 0.9752 at .01 = 1.29 *** at .001 = 1.68					Number of applications—no significant difference L.S.D.—Treatment at .05 = 22 ** at .01 = 29				

much the same for weight per sprout, an indication of size of sprout, as it was with number of sprouts.

Discussion

Spray applications of the methyl ester of naphthaleneacetic acid and of indoleacetic acid resulted in an increase in the petiole content of nitrate nitrogen, phosphorus, and potash, compared with those from unsprayed plants. Applications of sodium 2,4-dichlorophenoxyacetate resulted in increased petiole content of nitrate nitrogen at the second sampling and of potassium at the first date of sampling. STUART (11) found that large amounts of nitrogen and carbohydrates moved from other portions of bean plants to the portion which had been treated with indoleacetic acid. Naphthaleneacetic acid applications increased the potassium content of petioles at the first period of sampling. Application of none of the growth substances resulted in a significant decrease, compared with untreated plants, in petiole content of any of the five mineral nutrients.

There is some evidence that applications of certain growth substances to plants result in mobilization of certain nutrients and metabolized products in the area of application. In the present experiment possibly application of growth substances to the leaves resulted in an accumulation of certain nutrients in the petioles.



FIGS. 3–4.—Fig. 3, six tubers from control plants (unsprayed), showing amount of sprout growth after 3 months' storage at 50° F.; removed sprouts from six similar tubers. Fig. 4, seven tubers from plants sprayed three times during growing season with methyl ester of α-naphthaleneacetic acid, showing sprout growth and typical "pitted-scab-like" surface injury; removed sprouts from seven similar tubers.

HAMNER (4) found that relative percentage contents of calcium, phosphorus, and nitrogen in the bean plant were increased by the presence of naphthaleneacetamide in the nutrient solution. This may have been apparent rather than real in relation to other components, for SWARTZ (12) published as follows: "A significant decrease in total dry weight of the Marigold plants supplied with naphthaleneacetic acid (0.01 mg. per liter) indicates a retarding effect of the acid on growth." This agrees with the results of our experiment, in which yields of tubers were significantly reduced by the use of the methyl ester of NA.

There does seem to be some disagreement among workers using growth-regulators. For instance, LAUDE (5), working with the Red Kidney bean, *Phaseolus vulgaris*, found that, under certain levels of nutrition, increases as great as 12.5% in total plant dry weight were obtained by the use of low concentrations of indoleacetic acid in the nutrient solution.

The only treatment which resulted in any significant difference in yield compared with untreated plants was the application of the methyl ester of NA. The decrease in yield may have been due to injury to the leaves, particularly from the early applications, or to some alteration in the metabolism of the plant which resulted in accumulation of certain constituents in the leaves and stems rather than in the tubers.

That the methyl ester of NA sprayed on leaves and stems is translocated to the tubers is indicated by the fact that most tubers produced by such plants had injury similar to pitted scab on their under surfaces and to the fact that sprout growth was retarded in tubers from sprayed plants as it is on tubers treated in storage with the same substance. The dry weight of tubers from plants sprayed with the methyl ester of NA, as shown by specific gravity measurements, was significantly lower than that of tubers from any other treatment. SMITH and NASH (10) have shown that specific gravity of tubers may be influenced by many factors, such as variety, time of planting and of harvest, fertilizer treatments, light intensity, temperature during the latter portion of the growing season, maturity, and soil moisture. Immature tubers are of lower specific gravity than mature tubers. None of the above listed factors can be used to explain logically the lower specific gravity of tubers from plants receiving applications of the methyl ester of NA.

No significant differences in reducing-sugar content of tubers held 11 weeks at 40° F. could be found among any of the treatments. DENNY *et al.* (1) found no definite effect on the reducing-sugar content of the juice of tubers treated with the methyl ester of NA.

Spray applications of all the growth substances to plants in the field resulted in a significant decrease in amount of sprout growth of tubers subsequently stored at 50° F. The methyl ester of NA was much more effective in retarding sprout growth than any of the other growth substances. GUTHRIE (3) obtained inhibition of sprout growth of potatoes by treating whole or cut tubers with the potassium salt of indoleacetic acid, the potassium salt of naphthaleneacetic acid, and the methyl ester of NA.

Summary

1. Spray applications of the methyl ester of α-naphthaleneacetic acid (NA), sodium 2,4-dichlorophenoxyacetate, α-naphthaleneacetic acid, and indoleacetic acid were made to field-grown Irish potatoes at several stages of growth.

2. Applications of the methyl ester of NA at 10,000 p.p.m. at the rate of 55 gallons of mixture to the acre resulted in severe upward rolling of the leaves, with some of the leaves being injured severely. Rapid chemical tests of petiole tissues of plants sprayed with the methyl ester of NA showed a higher content of nitrate nitrogen, phosphorus, and potassium than in those of untreated plants. Yields were reduced, most tubers exhibited a "pitted-scab-like" injury, specific gravity of tubers was lowered, and sprout growth of tubers was inhibited as a result of spray applications of the methyl ester of NA. Applications of sodium 2,4-dichlorophenoxyacetate (10 p.p.m.), naphthaleneacetic acid (150 p.p.m.), and indoleacetic acid (150 p.p.m.), at rates varying from 55 to 110 gallons to the acre, had no visible effects on vine growth and did not affect yields of tubers, specific gravity of tubers, or reducing-sugar content of tubers after 11 weeks at 40° F. storage, compared with the untreated plants.

3. Petioles of plants sprayed with indoleacetic acid were higher in nitrate nitrogen, phosphorus, and potassium than those of unsprayed plants. Those sprayed with sodium 2,4-dichlorophenoxyacetate were higher in nitrate nitrogen and potassium; those sprayed with naphthaleneacetic acid were higher in potassium.

4. Tubers from all the sprayed treatments, after 3 months' storage at 50° F., had significantly less sprout growth than tubers from untreated plants.

DEPARTMENT OF VEGETABLE CROPS
CORNELL UNIVERSITY

LITERATURE CITED

1. DENNY, F. E.; GUTHRIE, J. D.; and THORNTON, N. C. The effect of the vapor of the methyl ester of naphthaleneacetic acid on the sprouting and sugar content of tubers. Contr. Boyce Thompson Inst. 12:253–268. 1942.
2. ENNIS, W. B., JR.; SWANSON, C. P.; ALLARD, R. W.; and BOYD, F. T. Effects of certain growth-regulating compounds on Irish potatoes. BOT. GAZ. 107:568–574. 1946.
3. GUTHRIE, J. D. Control of bud growth and initiation of roots at the cut surface of potato tubers with growth regulating substances. Contr. Boyce Thompson Inst. 11:29–53. 1939.
4. HAMNER, C. L. Physiological and chemical responses of bean and tomato plants to alpha naphthaleneacetamide and phenylacetic acid. BOT. GAZ. 103:374–385. 1941.
5. LAUDE, H. M. Combined effects of potassium supply and growth substances on plant development. BOT. GAZ. 103:155–167. 1941.
6. PEECH, M., and ENGLISH, L. Rapid microchemical soil tests. Soil Sci. 57:167–195. 1944.
7. SHAFFER, P. A., and SOMOGYI, M. Copper iodometric reagents for sugar determinations. Jour. Biol. Chem. 100:695–713. 1933.
8. SMITH, O. Retarding sprout growth of potatoes and root crops in storage. Down to earth 2:5–8. Dow Chemical Co., Midland, Mich. 1946.
9. SMITH, O.; ELLISON, J. H.; and MCGOLDRICK, F. Methyl ester of naphthaleneacetic acid as a selective herbicide in potatoes. (Unpublished data.) 1946.
10. SMITH, O., and NASH, L. B. Potato quality V. Relation of time of planting, time of harvest, and fertilizer treatment to composition and cooking quality. Jour. Amer. Soc. Agron. 34: 437–451. 1942.
11. STUART, N. W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. BOT. GAZ. 100:298–311. 1938.
12. SWARTZ, D. B. Effect of various growth-regulating substances upon several species of plants. BOT. GAZ. 103:366–373. 1941.

GROWTH OF FIELD CROPS IN SOIL TREATED WITH CHEMICAL GROWTH-REGULATORS

D. L. TAYLOR

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 581

Introduction

Reports of studies at Camp Detrick, Frederick, Maryland (7), emphasized the possibilities of the use of chemical growth-regulators as soil or spray treatments for controlling weeds in connection with general agricultural programs. Isopropyl-N-phenyl carbamate was found to inhibit markedly the development of seedlings of several common grasses, while most dicotyledonous species appeared to be little affected by such treatments. The possibility of using 2,4-dichlorophenoxyacetic acid to inhibit selectively the growth of weeds seemed likely considering the tolerances of the acid by a number of crops, especially grasses.

More recent studies (2, 5, 6) have dealt with treatment of soils, inactivation of seeds, seedling development, and the degree of persistence of activity of treatment and of viability of weed seeds in the soil. In some instances the soil was pretreated or treatments could be so construed.

It has been suggested that treatment of the soil before planting crops could afford a desirable variation of herbicidal technique (2, 6). Much remains to be defined, however, regarding the quantity, intensity, and timing of such treatment in relation to specific crops, the environment, and residual effects of treatments. Such effects could be reflected in the soil response, about which very little is yet described, in the development of seeds and seedlings, and in the later growth of plants on treated soil.

Tests involving treatment of the soil were conducted in an effort to obtain further information about weed control, persistence of effects of treatments, and the growth of field crops on soil pretreated with 2,4-dichlorophenoxyacetic acid (2,4-D) or isopropyl-N-phenyl carbamate (IPPC).

Material and methods

These field tests were conducted from April through September, 1946, at Lake Geneva, Wisconsin. Two areas of light to medium, loamy top soil were used. One soil had relatively low fertility, contained marl (crumbly clay and $CaCO_3$), and was alkaline, pH 7.8–7.9. The second soil, a medium-light silt loam, was of moderate fertility and had a pH of 6.9–7.0. During previous seasons both soils had been cultivated.

The 2,4-D was a white powder obtained from Dow Chemical Company. Isopropyl-N-phenyl carbamate (IPPC), which was secured from Sherwin-Williams Company, was a gray-white, granular solid (m.-p. 87°–89° C.).

The test crops, selected because of ease of cultivation and economic importance, were Red Kidney bean, Henry spring wheat, white mustard, Golden Bantam sweet corn, and Silver King (Wisconsin no. 7) white dent corn. Kidney bean and mustard are highly sensitive to 2,4-D and apparently insensitive to IPPC. Wheat and corn are sensitive to IPPC and relatively insensitive to 2,4-D, wheat being less so than corn.

Investigation

TEST 1

PROCEDURE.—On April 25 all weeds were removed from the test areas and 2,4-D or IPPC was applied at the rate of 0, 2, or 4 lb. per acre. Ten and five-tenths or 21 gm. of the chemicals, passed through a sixteen-mesh sieve, were thoroughly mixed into 50 lb. of lake sand, and passed through an eight-mesh sieve. Each mixture was broadcast evenly over test areas of 12 × 42 feet to supply the chemicals at the rate of 2 or 4 lb. per acre, respectively. All areas were raked over lightly once to mix the dressings into the top ½ inch of soil.

On each planting date all weeds which had appeared following treatment were removed from the parts of treated areas to be planted. Weeds were allowed to develop on the remainder of the treated areas, until these were required for plantings, in order to observe the degree of weed control on the unplanted ground.

Except in the plot treated with IPPC, the initial planting was made on April 25. Nine other plantings were made in all areas on the 7th, 14th, 21st, 28th, 31st, 35th, 38th, 45th, and 52d days (June 16) after treatment. At each planting twenty-five seeds each of corn, bean, and wheat were planted approximately 1.5 inches deep in separate rows, 20 inches apart.

Observations were made on the amount of, and time required for, emergence of shoots, the number and general condition of seedlings 30 days after planting, and the number and size of mature plants. Some corn plants were also harvested when they were at mid- to late-vegetative stage of development.

WEATHER.—Between April 25 and September 25, 16.3 inches of rainfall were recorded at the center of the experimental area. More than two-thirds of it fell during the first 2 months (table 1). Precipitation was one and a half and two times normal in May and June, respectively, but during the remainder of the period it was only 40% of the 44-year normal. Throughout the summer the extremes and means of daily temperatures were in general 1°–2° F. less than normal.[1]

RATE OF SEEDLING EMERGENCE.—When plantings were made within a week after the soil was treated with 2,4-D, the time required for emergence of seedlings from half of the corn or wheat seeds was 1 or 2 days, respectively, more than for the controls; for beans, 12–16 days (fig. 1). The 2-lb. treatment affected wheat and bean seedling emergence in successive plantings for 5–6 weeks. This persistence effect was irregular and less than 5 weeks in duration on emergence of corn seedlings for both 2- and 4-lb. treatments but lasted about 7 weeks for wheat and beans in soil pretreated with 4 lb. of 2,4-D per acre. Thus the duration of inhibitory residual effects was somewhat proportional to initial rate of treatment.

The IPPC treatment had slight and irregular, if any, effect on the emergence of bean, but markedly decreased and slowed the emergence of the cereals (fig. 1). Again corn appeared less sensitive than wheat. Not until seed was planted 4 weeks after treatment did 50% emergence of wheat occur, and then emergence of the much-stunted seedlings was delayed 7–10 days in comparison with the controls. The number of emerging corn seedlings, which were delayed for 8–20 days depending on the interval between soil

[1] The author is indebted to the Yerkes Observatory of the University of Chicago, Williams Bay, Wis., and to Miss MARY CALVERT for information concerning average rainfall and temperature and for data on temperatures for 1946.

treatment and planting, was less than half that of the control if plantings followed within 2 weeks after treatment.

SEEDLING NUMBER AND CONDITION.—Observations were made on the general condition and numbers of seedlings 30 days old in relation to time of emergence of seedlings and survival of plants to maturity. Beans, when planted up to 5 weeks following application to the soil of 2,4-D at the 2-lb. rate, had shoots slightly smaller than the controls and showed distinct symptoms of 2,4-D effect on the leaves—especially attenuated, abnormally shaped primary leaves. Leaf symptoms which developed when seed was planted 45 days after the 4-lb. treatment were soon outgrown.

No growth-regulator treatment of soil appeared to induce an increase in the size of seedling shoots of any planting of beans. Inhibition of shoot was distinctly visible on seedlings 30 days old from plantings made within 4 weeks after treatment with 2,4-D. The tops of control plants appeared to be two or more times as large as those for plantings made within 2 or 6 weeks following application of 2 or 4 lb., respectively, of 2,4-D per acre.

Development of root systems was curtailed and abnormal when beans were planted within 2 weeks after treatment of soil with 2,4-D. In most instances only curved hypocotyls emerged from the soil. Normal-appearing roots often developed at subsurface cotyledonary nodes of such plants, and a few shoots emerged very tardily from the soil. Inhibited misshapen primary root systems of some such plants were completely elevated by subsequent straightening of the hypocotyls.

In plantings of beans made within 2 weeks after treatment the populations of seedlings were smaller, in proportion to

TABLE 1

SUMMARY OF DATA ON RAINFALL AND TEMPERATURE AT LAKE GENEVA, WISCONSIN, DURING MAY THROUGH SEPTEMBER, 1946

Rainfall and temperature	May	June	July	August	September
	Rainfall (in inches)				
Recorded, 1946	4.06	7.35	1.91	1.74	1.19
Difference from normal	+ 1.67	+ 3.69	− 1.46	− 2.04	− 2.64
	Temperature (in °F.)				
Mean recorded, 1946	55.5	66.0	72.6	68.2	64.0
Difference from normal	− 2.0	− 1.6	− 0.3	− 2.7	+ 0.5
Mean maximum recorded, 1946	66.5	76.5	83.6	79.3	76.0
Difference from normal	− 2.1	− 1.9	− 0.5	− 2.5	+ 2.2
Mean minimum recorded, 1946	44.5	55.4	61.6	57.0	52.0
Difference from normal	− 2.0	− 1.6	− 0.3	− 2.7	+ 0.5

the rate of application of 2,4-D, than in untreated soil. Seedlings, 30 days old from plantings made within 3 weeks after the soil was treated with 2,4-D, were less able to survive to maturity than control seedlings (fig. 2).

Pretreatment of soil with 4 lb. of 2,4-D per acre reduced the size of populations of wheat seedlings distinctly less than of beans (fig. 2). The decreases of wheat were less in soil treated with 2 lb. of the acid per acre. The percentage of survival of wheat seedlings to maturity from plantings made within 4 or 5 weeks after soil treatment was lowered slightly by both rates of application.

The sizes and subsequent survival of populations of corn seedlings indicated that, of the crops tested, corn was least inhibited by the 2,4-D treatments. When plantings were made 4 or 5 weeks after pretreatment of soil with either rate of application, shoots appeared normal in size after 30 days of growth. In plantings made more than 5 weeks after treatment, seedlings 30 days old seemed normal and somewhat larger than in untreated soil.

Treatment of soil with IPPC did not appear to influence markedly the survival of bean seedlings (fig. 2). With wheat, however, the sizes of populations and the inability of many seedlings to survive if plantings were made within 5 weeks after treatment reflected the distinct inhibitory effect of IPPC on this crop. With corn, by the end of 30 days, populations were reduced 75% or more if planted within 2 weeks after treatment (fig. 2), but seedlings developed from three-fourths of the corn seed planted 3 weeks after treatment. With later plantings, the treatment appeared to have no consistent effect on corn seedling populations or their survival. Subsequent survival of 30-day-old seedlings was greater for corn than for wheat when plantings were made within 3 weeks after treatment.

WEED RESPONSES.—Growth of weeds was distinctly reduced by pretreatment of soil with 2,4-D. IPPC did not influence the growth of dicotyledonous weeds and did not appear distinctly effective against yellow bristle grass, *Setaria lutescens* (Weigel) F. T. Hubb., or crab grass, *Digitaria sanguinalis* (L.) Scop.—the major weed grasses present.

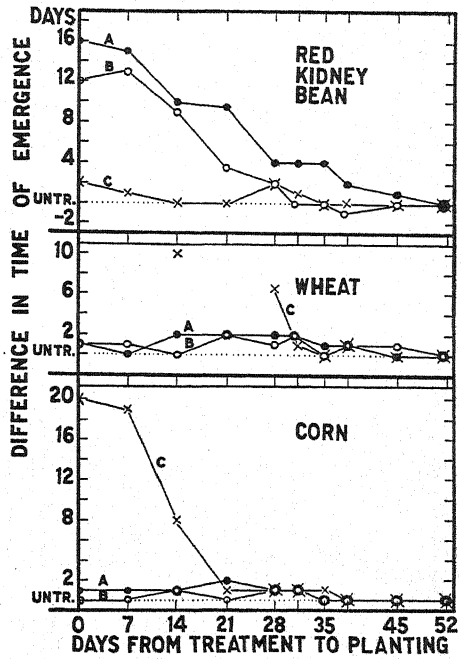


FIG. 1.—Differences in time required for emergence of bean, wheat, or corn seedlings from half of seeds planted in untreated soil and in soil pretreated with (*A*) 4 lb. of 2,4-D per acre, (*B*) 2 lb. of 2,4-D per acre, or (*C*) 4 lb. of IPPC per acre. Wheat did not emerge when plantings were made immediately, 7 days, or 21 days after soil was treated with IPPC. Numbers of days required for emergence of seedlings from successive plantings in untreated soil were: bean, 18, 13, 11, 11, 11, 9, 9, 8, 9, 10; wheat, 12, 10, 7, 6, 6, 5, 7, 5, 5, 7; and corn, 11, 8, 7, 8, 6, 6, 8, 6, 7, 9, respectively.

All the planted areas were again weeded during the third week in June and the last week in July because weeds were ex-

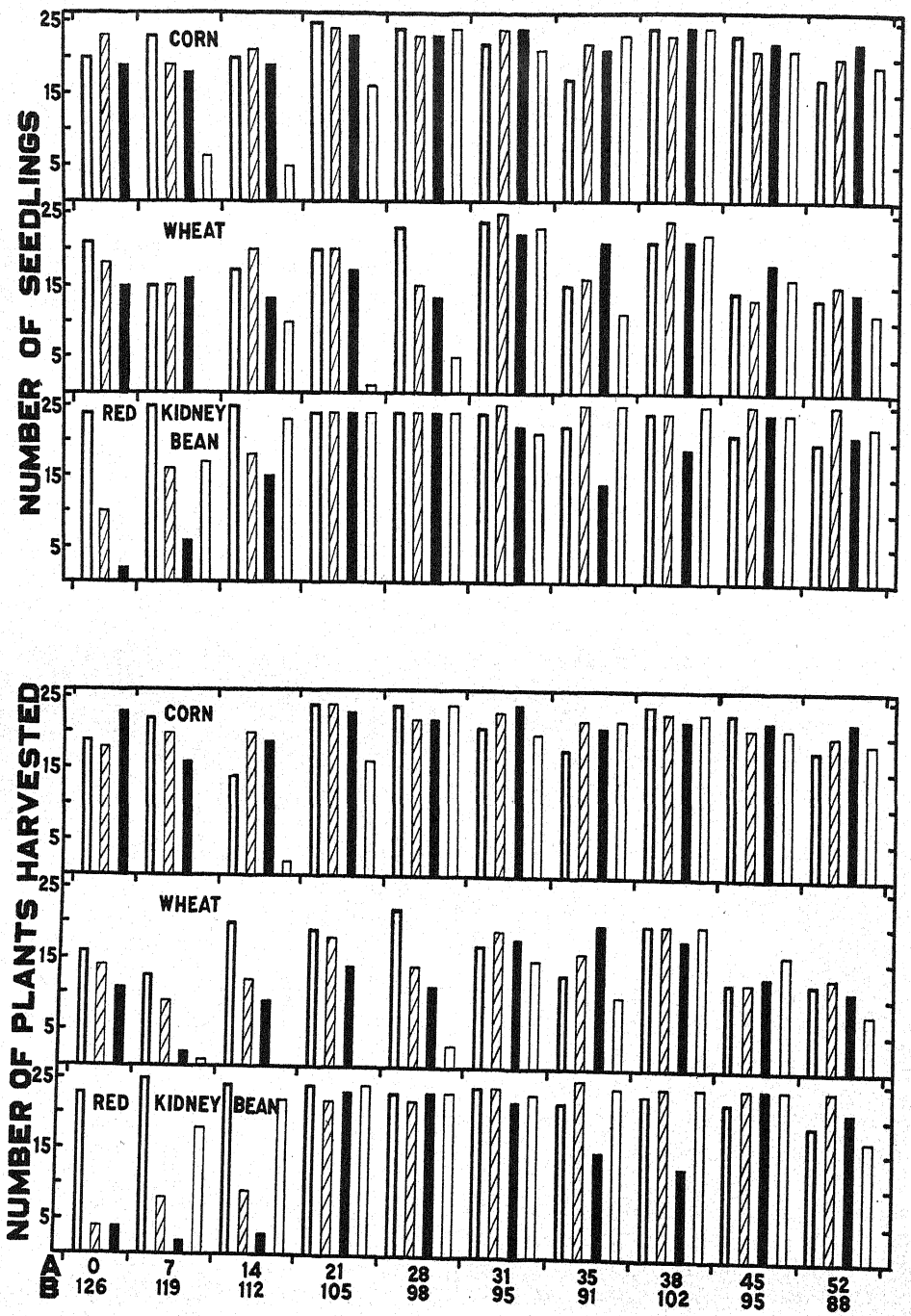


FIG. 2.—Numbers of bean, wheat, and corn plants present at 30 days (*upper*) or harvested (*lower*) when twenty-five seeds of each were sown at intervals up to 52 days following treatment of soil with 2,4-D or IPPC. Four bars which represent each planting denote soil treatments: *left bar*, untreated soil; *hatched bar*, 2 lb. of 2,4-D per acre; *black bar*, 4 lb. of 2,4-D per acre; *right bar*, 4 lb. of IPPC per acre. Numbers along line *A* indicate time in days between treatment of soil and planting. Age in days of plants harvested from different plantings is shown on line *B*.

cessive in the untreated and in the IPPC-pretreated areas.

WEIGHT OF VEGETATIVE TOPS OF CORN.—During June and July, when conditions prevailed which were favorable for the growth of corn, it became apparent that plants in soil pretreated with 2,4-D were exceeding others in size. On August 1, when 98–46 days old, one-half of the plants from each planting were harvested. The silks were then dry on corn planted within 2 weeks after soil was treated and were functional on plants from the succeeding four plantings. Corn planted 38 or 45 days after soil treatment was tasseled and coming into tassel, respectively. In the last planting the tassels had not emerged.

In the first six plantings (made within 31 days after soil treatment) the fresh weight of tops of corn grown in soil treated with 2 lb. of 2,4-D was greater than the controls by 6–58% (table 2). In such soil the weights of tops of later plantings were less regularly related to treatment conditions and approximately were equal to the controls or exceeded them by up to 50%. When maximum drought conditions prevailed during the period when major growth normally would have occurred (plantings 6 and 7), the weights of shoots were most nearly equal for plants grown in treated and untreated soil.

Corn planted 2 weeks or more after treatment of soil with 4 lb. of 2,4-D per acre had stems which were 12–45% heavier than plants grown in untreated soil. As the length of the period between soil treatment and planting date increased, the weights of the shoots of such treated plants tended to exceed by greater amounts those grown in soil treated with 2 lb. per acre. It did not appear that 2,4-D had otherwise altered the general developmental sequence of corn.

Pretreatment with IPPC resulted in shoots of smaller weight as compared with controls when plantings were made up to about 5 weeks after soil treatment. The appearance of tassels and silks appeared to be delayed for 1–2 weeks. Later plantings were not so influenced by the soil treatment.

POPULATION AND WEIGHT OF TOPS OF MATURE PLANTS.—Mature crops at ages of 91 days, or more, which had been planted within 5 weeks, or less, after the time the soil was treated were counted and harvested on August 29. In order to allow them to mature, the later plantings were not harvested until September 12, when they were 102–88 days old. At the time of harvest all beans had finished flowering and pods were full, wheat was past the "milk-dough" stage, and the ears of corn were filled out.

Some older leaves of wheat plants more than 90 days old had dried, and plants 15 weeks old were dry. During the growth period it appeared that flowering of beans growing in soil treated with 2,4-D had been delayed 10–14 days. Bean plants grown in soil pretreated with growth-regulators, especially with IPPC, remained green longer than controls.

Pretreatment of soil with 2,4-D reduced numbers of beans which survived to maturity compared with controls if plantings were made up to 2 weeks after treatment (fig. 2). This response occurred in wheat planted within 4 weeks of the time of treatment. The numbers in mature corn populations were limited only slightly, if at all, when plantings followed treatment of the soil with 2,4-D by 1 week (fig. 2).

When soil was pretreated with IPPC, numbers of bean plants were not limited compared with controls if plantings were made more than 1 week after treatment.

Numbers of corn or wheat plants were reduced if plantings followed this treatment within 3 or 4–5 weeks, respectively.

The fresh weight of tops of mature bean plants in soil pretreated with 2 lb. of 2,4-D per acre was lower than the controls for the first five plantings (table 3) and for the first six plantings in soil treated at the 4-lb. rate. Tops grown in soil treated with IPPC were heavier than the controls when plantings were made within 5 weeks after treatment. Bean plants grown in soil treated with 2,4-D were heavier and those in IPPC-treated soil were lighter than the controls when planted more than 5 weeks after the soil was treated (table 3).

Vegetative stems of corn and mature

TABLE 2

AVERAGE FRESH WEIGHTS OF TOPS OF VEGETATIVE AND MATURE CORN PLANTED AT INTERVALS UP TO 52 DAYS AFTER SOIL WAS TREATED WITH 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) OR ISOPROPYL-N-PHENYL CARBAMATE (IPPC)

Date of planting	Length of time (days)		Average weight of tops per plant			
	Treatment to planting	Growth period	Untreated soil (gm.)	Percentage difference* from tops in untreated soil when soil was pretreated with—		
				2,4-D		IPPC
				2†	4†	4†
	Vegetative					
April 25	0	98	632	+58	− 6	Not planted
May 2	7	91	613	+32	− 4	−27
May 9	14	84	649	+45	+21	−32
May 16	21	77	630	+ 6	+25	−42
May 23	28	70	605	+32	+27	−26
May 26	31	67	671	+ 8	+12	−27
May 30	35	63	731	−13	+19	−24
June 2	38	60	541	+23	+29	− 2
June 9	45	53	585	+ 2	+15	− 3
June 16	52	46	307	+50	+45	+15
	Mature					
April 25	0	126	644	+50	+17	Not planted
May 2	7	119	765	+27	+59	No harvest
May 9	14	112	835	+18	+12	−28
May 16	21	105	722	+50	+35	−45
May 23	28	98	881	+34	+ 2	−40
May 26	31	95	824	− 4	− 6	−16
May 30	35	91	743	− 8	− 5	−12
June 2	38	102	681	+ 3	+18	− 6
June 9	45	95	652	+15	− 3	−19
June 16	52	88	802	+ 5	− 2	−12

* Relative average weight of tops of corn grown in treated soils is calculated in relation to value of 100% assigned to average weight of top per plant of same age grown in untreated soil.

† Equivalent pounds per acre.

tops of wheat were lighter than the controls when planted less than 40 days after the soil was pretreated with IPPC. Mature tops of corn were lighter than the controls for all plantings in the IPPC-treated soil.

The tops of wheat plants were approximately equal to the controls in weight for plantings made 1 or 2 weeks following treatment of the soil with 2 lb. of 2,4-D per acre (table 3). Unavoidable loss of some dry mature parts of plants on treated soil may have accentuated the differences between their weights and those of the controls planted 2–6 weeks after treatment. Tops of wheat planted within 6 weeks after the soil was treated with 4 lb. of 2,4-D per acre were up to 75% lighter than those of controls, depending on the pretreatment period.

The tops of mature corn plants from plantings made within 3–4 weeks after application of 2,4-D were consistently heavier than controls by 12–59% (table 2). Stems from later plantings in treated and untreated soil were about equal in size, whereas the vegetative tops of such plants in soil pretreated at the 4-lb. rate had been heavier than controls. Vegetative shoots produced from plantings made within a week after application of this treatment had been about equal to control plants in weight.

TABLE 3

AVERAGE FINAL FRESH WEIGHTS OF TOPS OF BEAN AND WHEAT PLANTS GROWN FOR PERIODS OF 88–126 DAYS AFTER SEEDS WERE SOWN AT INTERVALS UP TO 52 DAYS FOLLOWING PRETREATMENT OF SOIL WITH 2,4-D OR IPPC

Date of planting	Length of time (days)		Average weight of tops per plant							
					Percentage difference* from tops in untreated soil when soil was pretreated with—					
					2,4-D				IPPC	
					2†		4†		4†	
	Treatment to planting	Growth period	Untreated soil (gm.)							
			Bean	Wheat	Bean	Wheat	Bean	Wheat	Bean	Wheat
April 25.....	0	126	183	6.8	−26	−19	−15	−59	Not planted	Not planted
May 2.....	7	119	202	4.5	−33	− 2	−74	−78	+41	No stand
May 9.....	14	112	193	5.0	−54	+20	−80	−62	+22	No stand
May 16.....	21	105	172	8.3	−21	−35	−69	−41	+ 8	No stand
May 23.....	28	98	133	11.6	−11	−33	−24	−28	+44	−38
May 26.....	31	95	122	9.3	+19	−14	−32	−42	+33	+20
May 30.....	35	91	132	10.9	+33	−26	+20	−44	+22	−16
June 2.....	38	102	127	13.5	+15	−22	0	−42	−13	−21
June 9.....	45	95	175	6.5	+24	− 1	− 9	0	−13	+17
June 16.....	52	88	207	7.9	+23	−14	+42	+25	−20	− 9

* Relative average weight of tops of plants grown in treated soils is calculated in relation to value of 100% assigned to average weight of top per plant of same age grown in untreated soil.

† Equivalent pounds per acre.

TEST 2

Late in May, when shoots of corn plants growing in soil pretreated with 2,4-D began to appear larger than the controls, the question of the magnitude of the effect of weed competition arose.

A second test was initiated to attempt to determine whether the apparent greater growth of corn in treated soil was due to the competition between corn and weeds in the untreated area or to an actual stimulation of growth resulting directly from treatment of the soil.

PROCEDURE.—The methods were in most ways similar to those used in test 1. All weeds were removed from an area of silt-loam soil which did not contain marl and which had a pH of 6.9–7.0. This area was divided into three parts, each 8 × 25 feet, by shallow trenches.

On June 2 two parts were treated with 2,4-D at rates of 1 or 3 lb. per acre. The third part was left untreated. Plantings were made in one-third of each area 20, 30, or 40 days after treatment. Immediately before each planting all weeds were removed from all treated areas. After the planting on the 20th day, weeds were not removed from the areas planted at that time, but the remaining parts of the treated areas were kept free of weeds throughout the test period.

At each planting 64 grains of sweet corn and 30 white mustard seeds were sown in each area. Such mustard seedlings as developed were removed 20 days after planting by which time their response had indicated any residual inhibitory activity of the soil treatments. The amount and time of emergence of seedlings and the growth of weeds in the areas treated 20 days before planting were observed. Half of the corn from each planting was harvested when 48 (late vegetative) or 72 days old (fully reproductive).

RESULTS.—By 10 days after mustard seeds were sown in soil pretreated for 20 days with 1 or 3 lb. of 2,4-D per acre, emergence was 13% or 7%, respectively, compared with more than 75% emergence in untreated soil. Seedlings in treated soil were much stunted. No further emergence from treated soil occurred. Less than 50% of the mustard seed produced seedlings, except when sown 40 days after treatment. Their emergence was delayed for 1–2 days, and strong symptoms of effect of treatment with 3 lb. of 2,4-D were evident in this last planting.

The numbers of corn seedlings which emerged from the 20-day planting in pretreated soils were equal to those in the controls, but emergence was delayed for a day. Similar delay resulted for corn planted 30 days after soil had been treated with 3 lb. of 2,4-D per acre. In the 30- and 40-day plantings 15–35% more seedlings emerged during the period of drought from pretreated than from untreated soil. Survival of seedlings to maturity did not appear to be influenced by the treatments.

Suppression of the number and size of weeds, which principally were purslane, *Portulaca oleracea* L., chickweed, *Alsine media* L., and yellow bristle grass, in areas treated with 1 or 3 lb. of 2,4-D per acre was 85% or 95%, respectively, compared with weeds in untreated soil. To some extent reduction was proportional to rate of treatment, although all areas pretreated with 2,4-D were remarkably free from weeds.

Three weeks after the first planting, corn plants in soil treated with 3 lb. of 2,4-D per acre appeared as large as the controls, and those in soil treated with 1 lb. appeared larger than controls. Plants in untreated soil appeared to grow more slowly than those in treated soil as the growth period progressed. There was no indication that 2,4-D treatments influenced the time of appearance of tassels or silks.

Corn of the 20- and 30-day plantings was in tassel, just showing silks, and the

40-day planting was only in tassel when 48-day-old plants of each of these lots were harvested. When plants 72 days old were harvested, they were green and ears were edible. In the plantings made 30 days after treatment many ears of control plants were less well filled than on plants grown in treated soil.

Fresh weights of vegetative and reproductive tops (table 4, fig. 3) grown in treated soil were distinctly greater than in the controls. When pretreatment was with 1 lb. per acre, the tops of 48-day-old plants from the 20-day planting were heavier than those in soil treated with 3 lbs. per acre. This difference was half as great when the plants were 72 days old. For other plantings and treatments this relationship was reversed.

When plantings were made more than 20 days after treatment, differences in the intensity of treatment did not result in differences in the weights of tops with the exception of the 72-day plants from the 40-day planting. In the latter case treatment with 3 lb. of 2,4-D per acre resulted in larger plants. It is evident that stimulation in the growth of corn resulted from pretreating the soil with 2,4-D, whether weeds were allowed to grow and compete or were removed by cultivation.

TABLE 4

TOTAL YIELDS AND AVERAGE WEIGHTS OF TOPS OF CORN GROWN IN UNTREATED SOIL AND IN SOIL PRETREATED WITH 2,4-D 20–40 DAYS BEFORE PLANTING

Planting		Growth-regulator treatment (lb./acre)	Total harvest			Average fresh weight and % difference in average weight of tops per plant harvested at—			
						48 days		72 days	
Time after treatment (days)	Management		No. of plants	Weight and % difference in weight					
				Kg.	%	Gm.	%	Gm.	%
20	Not weeded	0	52	7.65	.....	132	.....	162	.....
	Not weeded	1	54	13.64	+78	211	+60	294	+81
	Not weeded	3	51	11.48	+50	180	+36	272	+68
30	Weeded	0	48	10.60	.....	153	.....	288	.....
	Weeded	1	46	15.73	+48	258	+69	425	+48
	Weeded	3	55	18.49	+74	250	+63	425	+48
40	Weeded	0	34	4.74	.....	107	.....	172	.....
	Weeded	1	38	5.87	+24	146	+36	163	− 5
	Weeded	3	47	8.05	+70	145	+35	197	+15

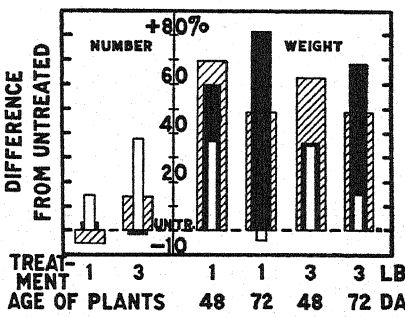


FIG. 3.—Relative differences from controls (*Untr.*) in numbers of plants harvested and in average fresh weights of 48- or 72-day-old tops from corn planted 20 days (*solid bars*), 30 days (*hatched bars*), or 40 days (*white bars*) after soil was treated with 1 or 3 lb. of 2,4-D per acre.

Discussion

In these field tests a desirable inhibition of weed growth resulted, and re-

sponses of test crops indicated that residual activity of IPPC or 2,4-D persisted in soil approximately 5 or 7 weeks, respectively. Some inhibition of the growth of kidney beans or wheat occurred in plantings made up to 5 or 6 weeks, respectively, after the soil was treated with 2,4-D. Bean shoots were slightly heavier than controls if plantings were made $4\frac{1}{2}$ weeks, or more, after the soil was treated with 2,4-D or within 5 weeks after soil treatment with IPPC. The rate and amount of growth of corn was distinctly stimulated when soil was pretreated with 2,4-D.

Dicotyledonous weeds were almost completely eliminated throughout the growing season on neutral and alkaline, medium-light, field soil which was treated at the start of the season with 1–4 lb. of 2,4-D per acre. IPPC did not influence the growth of such weeds. The growth of yellow bristle grass and crab grass was slightly greater in untreated soil than in soil treated with 2,4-D or IPPC during periods which lasted not more than 3–4 weeks after treatment.

In general, the development of beans and mustard was more inhibited than that of wheat and corn in proportion to intensity of 2,4-D treatment (treatment rate × time interval between treatment of soil and planting); IPPC had more effect on the cereals. The responses of test crops in soil treated with 4 lb. of 2,4-D per acre were, for the most part, parallel to but delayed for about 2 weeks compared with those for the 2-lb. treatment.

TABLE 5

LENGTH OF TIME (WEEKS) REQUIRED BETWEEN TREATMENT OF SOIL AND SOWING OF SEED IN ORDER THAT PLANTS IN SOIL TREATED WITH 2,4-D AND IN UNTREATED SOIL WERE SIMILAR CONCERNING VARIOUS CRITERIA

Criterion	Treatment (lb./acre)	Crop tested		
		Bean	Wheat	Corn
Time required for emergence of seedlings	2	$4\frac{1}{2}$	5	5
	4	7	6	3–5
Number of seedlings emerged during 30 days after planting	2	3	1	0
	4	4–6	$4\frac{1}{2}$–5	3–4
Visible symptoms of deleterious effects on tops	2	$5\frac{1}{2}$	$4\frac{1}{2}$	4–$4\frac{1}{2}$
	4	$7\frac{1}{2}$	$6\frac{1}{2}$–$7\frac{1}{2}$	5
Number of mature plants	2	3–4	$4\frac{1}{2}$	1–2
	4	4–6	6	3–4
Survival of seedlings after 30 days	2	3	3	1
	4	3	4–$4\frac{1}{2}$	2
Weight of mature tops	2	$4\frac{1}{2}$	5–6	0
	4	5	5–6	1–2

Table 5 summarizes the duration of inhibitory responses in test 1. The combined effects of strong inhibition of root systems of bean, abnormal shoot growth of which was appreciable, and drought condition during the period when major growth normally occurred seem the most plausible explanation for the small sizes of populations (fig. 2) and the low weight of mature plants (table 3) from plantings made 5 or 6 weeks after treatment of soil

with 4 lb. of 2,4-D per acre. Data on other crops for these plantings are not contradictory to this explanation. The relatively large weights of mature shoots of wheat for plantings made 1–2 weeks after treatment with 2,4-D may in part have been due to unavoidable loss of parts of control plants which were completely dry before harvest. Relatively heavy shoots from the last planting in soil treated with 4 lb. per acre may in part have resulted from the effect of 2,4-D treatment in slightly prolonging the green condition of plants.

Responses likely resulted from the influence of the calcium salt of 2,4-D formed by reaction with marl ($CaCO_3$) in the soil which was low in organic matter. It should be recalled in considering residual activity that 11.5 or 8.5 inches of rain fell during 6–8 weeks immediately after treatment of soil in tests 1 or 2, respectively. Also, the temperature during the first 2 weeks of test 1 was unfavorable for growth of kidney beans and corn.

On the basis of the criteria described, some residual inhibitory activity of 2,4-D lasted in the soil for about 50 days compared with 95% survival of soybeans which DeRose (1) reported for plantings made 90 days after 2,4-D was applied at the rate of 50 lb. per acre on silt-loam soil. In greenhouse tests Mitchell and Marth (6) found normal emergence of barley but "reduced" emergence of mustard in a soil low in organic matter which had been treated with 4 or 14 mg. of 2,4-D per pound and then stored dry for 1 or 6 months, respectively.

Table 6 summarizes the duration of inhibitory effects of soil treatment with IPPC. There was little consistent effect of IPPC treatment on later plantings of corn or wheat. The green condition of wheat plants was slightly prolonged.

It appeared that temporary and inhibitory effects of IPPC activity persisted about 5 weeks in the soil compared with 7 weeks for 2,4-D when applications were made at the rate of 4 lb. per acre. This relation corresponds with that found in greenhouse tests with non-alkaline silt loam and in field tests where applications were made at the rate of 50 lb. per acre (1).

Dry leaves were almost lacking at harvest time on bean plants, 8–44% heavier than controls, for plantings made within 5 weeks after soil was pretreated with IPPC. The period during which this response was evident corresponds well with the period and intensity of residual activity indicated by other criteria. Prolongation of the green condition of tops of beans for plantings made $4\frac{1}{2}$ weeks or more after treatment of soil with 2,4-D must have contributed to the relatively heavier tops of these plants. However, such plants were visibly slightly larger and their lower stems thicker than those of control plants. Rather distinct apparent increases over the controls in the size of vegetative plants have been observed for kidney beans planted in

TABLE 6

LENGTH OF TIME (WEEKS) REQUIRED BETWEEN TREATMENT OF SOIL WITH 4 LB. OF IPPC PER ACRE AND SOWING OF SEED IN ORDER THAT PLANTS IN TREATED AND IN UNTREATED SOIL WERE SIMILAR CONCERNING VARIOUS CRITERIA

Criterion	Crop tested		
	Bean	Wheat	Corn
Time required for emergence of seedlings	1–2	5–6	4–5
Number of seedlings emerged during 30 days after planting	2	5	4–5
Visible symptoms of deleterious effects on shoots	1–2	$5\frac{1}{2}$	$4\frac{1}{2}$
Number of mature plants	2	5	4
Survival of seedlings after 30 days	0	4–5	3
Weight of mature tops	0	5	5–6

soil which had been in moist warm storage for 1–2 months following application of 2,4-D (4).

The most striking response was the distinctly greater growth of corn in soil pretreated with 2,4-D than in untreated soil (tables 2–4). Compared with controls, tops were distinctly (13%) lighter only for one planting and were distinctly heavier (12–60%) for a majority of the twenty plantings made in treated soil in test 1. Stimulation was most consistent when plantings were made within 1 month after treatment and was greatest for plantings which followed within 2 weeks after soil was treated with 2 lb. of 2,4-D per acre. The vegetative stems of corn for early sowings in soil treated with 4 lb. per acre weighed less than those in soil treated at the 2-lb. rate but were practically equal to controls. In soil treated at the 4-lb. rate, vegetative corn plants were consistently heavier (12–45%) than controls in later plantings. HANKS (3) found an apparent stimulation of root elongation when the corn bio-assay technique was used to measure residual activity in leachates obtained from different soils which had been held in moist storage for 2–6 weeks after treatment with the acid or its calcium salt. The response was most consistent for sandy loam stored for 2 weeks or alkaline silt loam stored for 6 weeks.

The length of the period of vegetative growth and the total numbers of corn plants available for harvest did not appear distinctly influenced by 2,4-D treatment. Survival of plants which were most subjected to drought condition (40-day planting in test 2) appeared to be slightly greater in soil pretreated with 2,4-D than in untreated soil (table 4, fig. 3).

Mature stems of corn grown in treated soil were 12–59% heavier than controls for plantings made within 1 month after treatment in test 1 and 15–60% heavier in weed-free soil for plantings made 30 or 40 days after treatment in test 2. Weed competition in untreated soil was undoubtedly an influencing factor in test 1 and for the 20-day plantings in test 2. In the latter case the ground was not weeded, and lush weed growth occurred in untreated soil, while treated areas remained relatively free of weeds.

On the bases of weight of vegetative or mature tops, growth of corn in treated soil was greater or more rapid than the controls when plantings were made 30 days after treatment; when made 40 days following treatment, the difference was lessened (table 4). Control plants grew faster, or more, during the latter part of the growth period than plants in treated soil when plantings were made more than 4 weeks after treatment in test 1 or when plantings were made 30 days or more after treatment in test 2.

It seemed that more rapid growth occurred in soil treated with 2,4-D but that final weights of plants for later plantings were more nearly equal in untreated and treated soil. This is clearly evident for the 40-day planting in test 2. Apparent stimulation of growth was consistent and permanent in appreciable amount when corn was planted 30 days or less after treatment of the soil with 2,4-D.

Changes in the amount of crop responses which indicated deleterious or inhibitory influence of soil treatments with 2,4-D seem quite compatible with a progressive decrease of residual toxic activity during 7 weeks. This occurrence in combination with a progressive decrease in a residual stimulating influence could account, at least in part, for the relatively greater growth during the latter part of the growth period by control plants for later plantings and likewise for rela-

tively greater growth of corn for earlier plantings in soil treated with 4 lb. of 2,4-D per acre.

Viewed as a whole, the results of these tests strongly indicate that with proper management of treatment a combination of weed control with desirable stimulation of crop growth might be possible.

Summary

1. In two field tests 2,4-dichlorophenoxyacetic acid (2,4-D) and isopropyl-N-phenyl carbamate (IPPC) were applied as top dressings in dry form at rates from 1 to 4 lb. per acre on neutral or alkaline loam soils at the start of the growing season. Successive plantings of Red Kidney beans, mustard, wheat, field corn, and sweet corn were made at intervals up to 52 days after the time of treatment of the soil.

2. Desirable control of weeds during the growing season resulted from treatment with 2,4-D, while IPPC did not influence dicotyledonous weeds and had only slight temporary inhibitory effect on weedy grasses.

3. Activity of the 4-lb. treatment of these growth-regulators, which was evaluated on test crops by means of the rate and amount of their emergence, number and condition of 30-day-old seedlings, survival of seedlings to maturity, and the amount of growth of tops, appeared to persist in the soil 7 weeks for 2,4-D and 5 weeks for IPPC. The growth of corn indicated that residual activity of the acid lasted longer than 7 weeks.

4. Growth-regulator treatments slightly prolonged the green condition of kidney beans; IPPC similarly influenced wheat.

5. Weights of shoots of mature bean or wheat plants grown in untreated soil and in soil treated with 4 lb. of 2,4-D per acre were equal when plantings were made 5–6 weeks after treatment.

6. The weights of shoots of kidney beans were generally slightly heavier than controls if plantings were made more than 4 weeks after treatment of the soil with 2,4-D.

7. Shoots of corn grew more rapidly and were heavier in soil pretreated with 2,4-D than in untreated soil. This stimulation was considered in relation to weed competition and residual activity of treatment.

Department of Botany
University of Chicago

LITERATURE CITED

1. DeRose, H. R. Persistence of some plant growth-regulators when applied to soil in herbicidal treatments. Bot. Gaz. 107:583–589. 1946.
2. Hamner, C. L.; Moulton, J. E.; and Tukey, H. B. Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid on germination and development of seedlings. Bot. Gaz. 107:352–361. 1946.
3. Hanks, R. W. Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching. Bot. Gaz. 108:186–191. 1946.
4. Kries, O. Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime. Bot. Gaz. 108. 1947. (In press.)
5. Mitchell, J. W., and Marth, P. C. Effects of 2,4-dichlorophenoxyacetic acid on the growth of grass plants. Bot. Gaz. 107:276–284. 1945.
6. ———, and ———. Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107:408–416. 1946.
7. Special Projects Division, Chemical Warfare Service, Camp Detrick, Md. Studies on plant growth-regulating substances. Bot. Gaz. 107:475–632. 1946.

SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 582

ROBERT S. BANDURSKI

The use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a differential herbicide applied directly to soil has stimulated investigation of the factors involved in its retention in that medium. Measurement of the persistence of the herbicide in stored soil has been accomplished by seedling emergence tests (3), and its concentration in the leachates of treated soils has been determined by HANKS (1) using the bio-assay method of SWANSON (5).

A spectrophotometric method for the determination of 2,4-D was developed in connection with a study of its retention by various soil components and synthetic ion-exchangers placed in contact with aqueous solutions of the acid. The method may be applied to the quantitative determination of 2,4-D in aqueous or ether solutions and in extracts of sand, clay, peat, and soil, provided that the concentration of other compounds which absorb light in the ultraviolet region does not become prohibitive.

SYNTHESIS AND PURIFICATION OF 2,4-D

Since commercial grades of 2,4-D may contain small amounts of compounds having chlorine atoms substituted at places other than the 2,4 positions, it was considered desirable to prepare this reagent. The method of synthesis, though not the purification procedure, is essentially that of POKORNY (4). Equimolar concentrations of 2,4-dichlorophenol (Eastman Kodak–white label), monochloroacetic acid (Merck's), and an excess of sodium hydroxide were placed in a small quantity of water and heated to near-dryness on a steam bath. The residue was washed with several portions of 95% ethanol and then dropped into a solution of 5% hydrochloric acid. The hydrochloric acid was decanted, and the crystals of 2,4-D were washed repeatedly with water. They were then dissolved in a minimal amount of 80% ethanol, the solution filtered, and the acid recrystallized by slow evaporation of 75% of the solvent. The large pure white crystals were then dried at 60° C. *in vacuo*. The melting-point of the product was 132°–134° C., which is not in good agreement with the figure of 138° C. reported by POKORNY. Earlier attempts to purify 2,4-D of technical grade by repeated salt-acid cycles and crystallization always resulted in a product having a melting-point of 132°–134° C. In spectrophotometric analyses the position of the maxima and the value of the extinction coefficients for purified technical 2,4-D and for that prepared by the above procedure were the same, within the limits of experimental error.

PHOTOELECTRIC SPECTROPHOTOMETRIC ANALYSIS

The absorption spectra of 2,4-D in aqueous and ether solutions were determined with a Beckman Quartz Spectrophotometer, model DU, using a hydrogen discharge tube as the light source and silica sample cells. Diethyl ether was chosen as one of the two solvents tested because 2,4-D can be quantitatively ex-

tracted from aqueous solutions of low pH by shaking with ether, thus providing a simple means of concentration of soil extracts.

Figure 1 presents absorption data for 2,4-D in aqueous and ether solutions. The numerical value of the extinction coefficient, k, is plotted on a logarithmic scale along the ordinate, and the wavelength, in angstrom units, along the abscissa. Beer's law is used in the form

$$k = \frac{1}{cx} \log_{10} \frac{I_0}{I_x},$$

where k is the extinction coefficient; c is the concentration in milligrams per milliliter; x is the thickness of the solution layer in centimeters; I_0 is the intensity of radiant energy transmitted by the solvent-filled cell; and I_x is the intensity

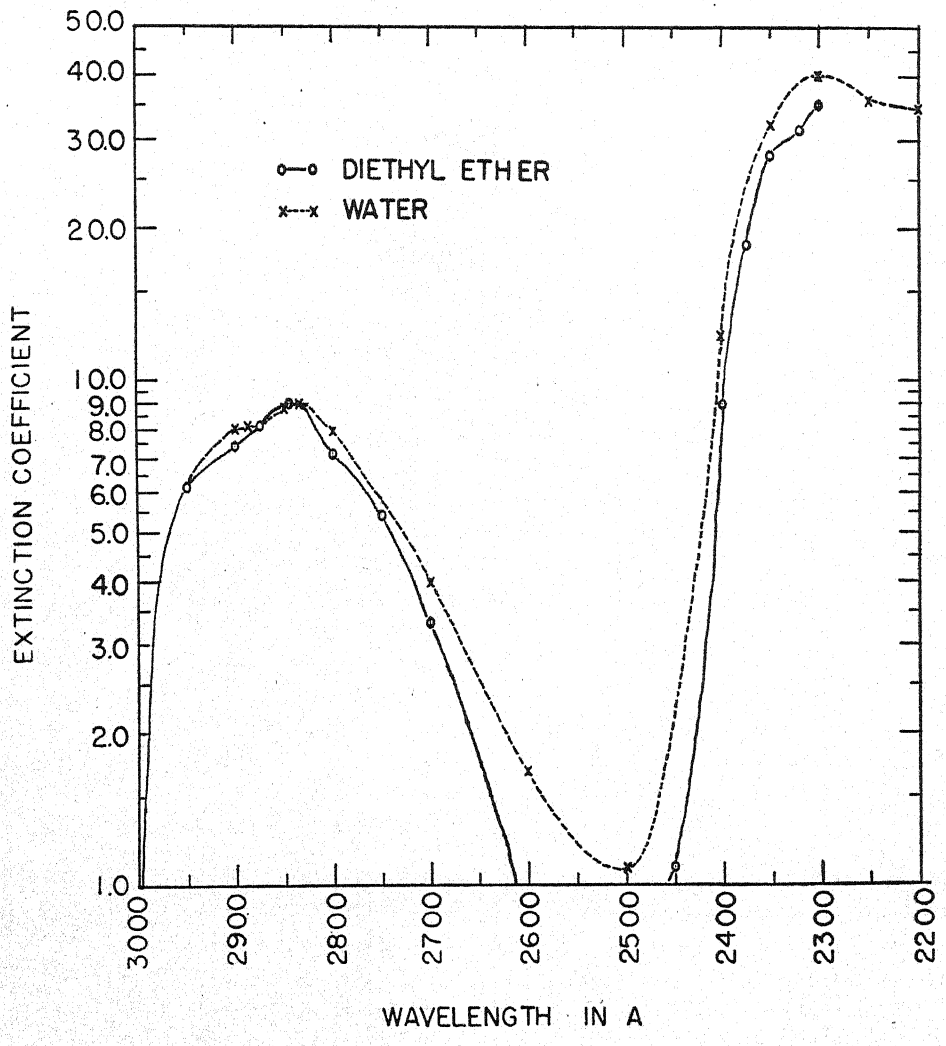


FIG. 1.—Absorption spectra of 2,4-dichlorophenoxyacetic acid in water and ether solutions

of radiant energy transmitted by the solution-filled cell.

Beer's law was found to hold within 5% limits over a concentration range of 6–250 γ/ml. The values of k for the acid in water, as measured at 2835 A and at 2300 A on a solution containing 25 γ/ml., were found to be independent of reaction over the range of pH 2.2–8.0; it is thus probable that Beer's law will hold for much greater dilutions. The absorption data are summarized in table 1.

TABLE 1

Solvent	Maxima (A)	Spectral region isolated, band width (A)	Extinction coefficient
Water....	2835	13	9.0
	2300	12	40.6
Ether.....	2845	17	9.0

TABLE 2

Wave-length (A)	Cell length (cm.)	Concentration (γ/ml.)	Computed concentration for maximum accuracy (γ/ml.)
2835.....	1.0	20–300	48.0
	5.0	3–50	9.6
	10.0	2–30	4.8
2300.....	1.0	5–70	11.0
	5.0	1–14	2.1
	10.0	0.5–7.0	1.1

Data are given in table 2 for the concentrations of 2,4-D which can be measured at the regions of maximum absorption with a minimum density of 0.2 and a maximum density of 2.8.

The computation of the optimum density for spectrophotometric work is discussed by LOOFBOUROW (2) following the method of TWYMAN and LOTHIAN (6). It is advisable to use a combination of concentration and cell length such that the values of $\log_{10}(I_0/I_x)$ fall within the range of 0.4–2.0 (7). If it is desired to work with solutions of very low densities, a calibration curve should be constructed.

Discussion

Though specific directions for measurement of 2,4-D in aqueous solutions containing other substances which absorb in the ultraviolet region cannot be given, owing to the wide variations in quantity and quality of the interfering substances, certain general recommendations may be made. In the case of extracts from clay, sand, and dilute aqueous extracts of peat, measurement of the concentration of the acid may frequently be made directly on the aqueous solution, after clearing of suspended matter. A blank solution, extracted under identical conditions with respect to pH, salt concentration, contact time, and temperature from a sample of the same medium not containing the acid, must be used. The reaction at which the solution density is determined can vary over the range of pH 2.2–8.0, although in strongly acid or alkaline solution the extinction coefficient varies, owing presumably to hydration effects.

If the concentration of substances which absorb in the ultraviolet region is too great to permit density determinations directly on the aqueous soil extract, recourse may be had to the following procedure. An aliquot of the solution is acidified (pH 3–4) and is extracted three times with a volume of diethyl ether equal to the volume of the aliquot. The volume of the ether may then be reduced, provided the volume of the ether extract of the blank is also reduced to the same point and made to the same volume.

Summary

1. A simple and accurate spectrophotometric method for the determination of 2,4-dichlorophenoxyacetic acid in aqueous and ether solutions is described.

2. The extinction coefficients for the acid in water at 2835 A and at 2300 A, and in ether at 2845 A, have been determined.

3. Certain chemical and instrumental precautions essential to quantitative results are discussed.

The author takes pleasure in acknowledging the aid of Dr. ROBERT A. WHITMORE, who first located the maxima in ether at approximately 2840 A and who gave many helpful suggestions during the course of the work.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. HANKS, R. W. Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching. BOT. GAZ. **108**: 186–191. 1946.
2. LOOFBOUROW, J. R. Physical methods for the identification and assay of vitamins and hormones. *In* Vitamins and hormones—advances in research and applications. 1:122. Academic Press, New York. 1943.
3. MITCHELL, J. W., and MARTH, P. C. Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. BOT. GAZ. **107**:408–416. 1946.
4. POKORNY, R. Some chlorophenoxyacetic acids. Jour. Amer. Chem. Soc. **63**:1768. 1941.
5. SWANSON, C. P. A simple bio-assay method for the determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solutions. BOT. GAZ. **107**:507–509. 1946.
6. TWYMAN, F., and LOTHIAN, G. F. Conditions for securing accuracy in spectrophotometry. Proc. Phys. Soc. (London) **46**:643–662. 1933.
7. VANDENBELT, J. M., and FORSYTH, J. Extinction coefficients of spectrophotometric standards as determined with the Beckman spectrophotometer. Ind. Eng. Chem. (Anal. Ed.) **17**:235–237. 1945.

ON THE LIFE-HISTORY OF VANILLA PLANIFOLIA

B. G. L. SWAMY

Vanilla planifolia Andr., a native of Central America, is grown in South India as a horticultural plant. Material for the present study was obtained from plants growing in a private garden. Serial microtome sections were stained with Heidenhain's iron-alum haematoxylin following customary methods.

Investigation

MICROSPOROGENESIS.—The wall of the microsporangium shows an epidermis (figs. 1–4), a fibrous endothecium (figs. 3–5), one, or sometimes two, middle layers, and one or two layers of tapetal cells which are very prominent in early stages. Only the endothecium persists in the mature anther (fig. 5).

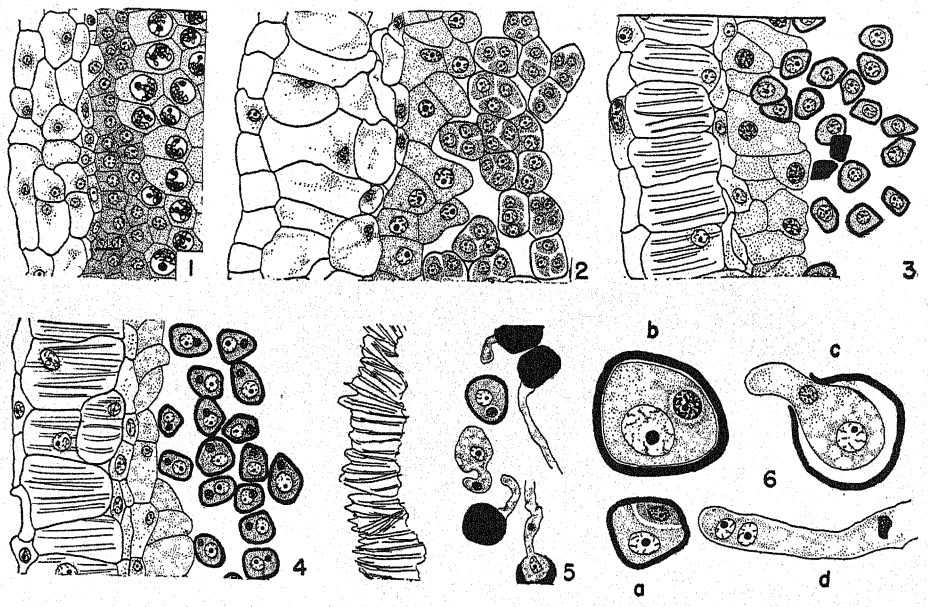
Just before the meiotic divisions, the polygonal microspore mother cells (fig. 1) begin to round up. The two divisions take place in rapid succession and are followed by inwardly directed furrows which result in the formation of the microspore tetrads (fig. 2). Unlike the condition in several other orchids, the microspores round up, develop a thick wall, and lie free in the anther sac (fig. 3).

The first division of the microspore cuts off a lens-shaped generative cell (fig. 6*a*), whose exact position in relation to the tetrad could not be determined,

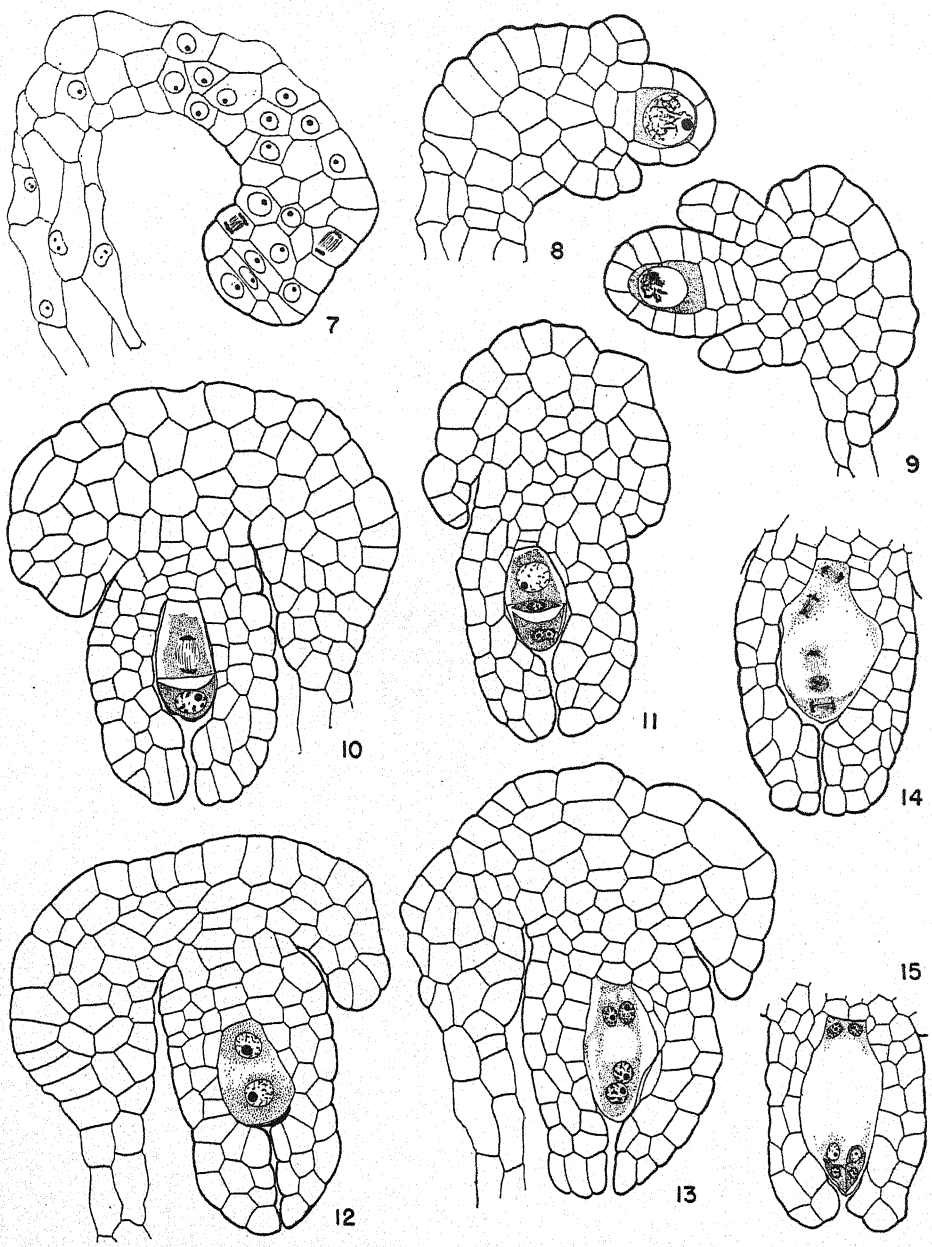
owing to the very early separation of the microspores. After a time the generative cell, with its very small and densely staining nucleus, is seen to lie inside the plasma of the tube cell (figs. 5, 6*b*).

The pollen is usually shed at the two-celled stage, but in many anthers observed the pollen grains had already germinated and put forth long pollen tubes (fig. 5). It is always the generative cell which moves into the pollen tube first, whatever may be its position in the pollen grain (figs. 5, 6*c*). During later stages, also, the degenerating tube nucleus can be seen behind the two sperm nuclei in the pollen tube (fig. 6*d*). Germination of pollen grains *in situ*, a curious feature in this plant, is connected with a tendency toward self-pollination, a subject which has been dealt with in detail elsewhere (4).

OVULE.—Differentiation of the ovules from the placental ridges starts only after pollination. In artificially pollinated flowers the archesporial cells become evident in the ovary 15–20 days after pollination. The placental ridges, which appear to be somewhat bilobed in a transverse section of the ovary, initiate divisions in the superficial cell layers and give rise to a number of papillae, each of which is three to four cells thick (figs. 7, 8). This is an important deviation from the great majority of orchids in which similar papillate outgrowths from the placenta occur at a corresponding stage but consist only of a unicellular filamentous row of five to six nucellar cells capped by the epidermis. It is also worth noting that in *Vanilla* the number of nucellar cells undergoes a further increase in the region below the



FIGS. 1–6.—Fig. 1, part of young microsporangium showing wall layers, tapetum, and sporogenous tissue. ×225. Fig. 2, same at microspore tetrad stage. ×225. Fig. 3, uninucleate microspores in anther loculus; note fibrous endothecium. ×225. Fig. 4, two-celled pollen grains. ×225. Fig. 5, germination of pollen grains within anther. ×225. Fig. 6*a*, generative and tube cells in pollen grain soon after division of microspore. ×900. Fig. 6*b*, pollen grain at time of shedding. ×900. Fig. 6*c*, germination of pollen grain. ×900. Fig. 6*d*, tip of pollen tube showing degenerating tube nucleus and two male nuclei. ×900.



FIGS. 7–15.—Fig. 7, nucellar papilla before differentiation of archesporial cell. ×225. Fig. 8, archesporial cell. ×225. Fig. 9, megaspore mother cell and origin of integuments. ×225. Fig. 10, dyad cells; chalazal in second meiotic division. ×450. Fig. 11, row of three cells; two megaspores and micropylar dyad cell. ×450. Fig. 12, two-nucleate embryo sac. ×450. Fig. 13, four-nucleate embryo sac. ×450. Fig. 14, division of all four nuclei (an exceptional case). ×450. Fig. 15, mature six-nucleate embryo sac. ×450.

archesporial cell, including the area at the base of the two integuments (figs. 9–13).

The inner integument differentiates more or less simultaneously with the archesporium (fig. 8) and is two- to three-layered (figs. 10, 11). The outer integument, which appears when the megaspore mother cell is undergoing reduction divisions, is also made up of three to four layers of cells and may be even five- to six-layered at the base (figs. 10, 35, 38). This is interesting, for in other orchids it consists of only one layer, or rarely two layers, of cells. In later stages the outer integument completely envelops the inner but is separated from the latter by a broad air space (figs. 35, 38).

MEGASPOROGENESIS AND FERTILIZATION.—The archesporial cell enlarges in size and functions directly as the megaspore mother cell (figs. 8, 9). The first meiotic division takes place in the usual manner. The micropylar dyad cell is always smaller and more darkly staining than the chalazal one (fig. 10) and only rarely undergoes further division. The chalazal dyad cell completes the second meiotic division but here also the micropylar daughter cell is smaller and more darkly staining than the other. Thus there originates a row of three cells, consisting of a uni- or binucleate dyad cell and two megaspores (fig. 11). Only the chalazal megaspore develops further. By the time of the meiotic divisions the nucellar epidermis has degenerated so that the developing embryo sac is in immediate contact with the inner integument (figs. 10, 11, 12).

The two-nucleate embryo sac (fig. 12) gives rise to the four-nucleate one. At first all four nuclei are of approximately the same size and appearance (fig. 13). The antipodal nuclei do not divide again, however (fig. 14 is an exceptional case), but stain deeply and persist as such up to the time of fertilization or slightly later (figs. 15, 17, 21). At the micropylar end the two synergids are formed by the division of one nucleus, and the egg and the upper polar nucleus by the division of the other nucleus (fig. 14). Thus the mature embryo sac (fig. 15) is six-nucleate, the upper polar nucleus alone taking part in the formation of the primary endosperm nucleus.

The pollen tube penetrates through the micropyle to enter the embryo sac. During this process one of the synergids is disorganized. One sperm nucleus fuses with that of the egg and the other with the upper polar nucleus, which lies close by (fig. 16).

ENDOSPERM.—The post-fertilization development presents some interesting features. Figure 16 shows a fertilized embryo sac. The primary endosperm nucleus, which is diploid, moves to the center of the embryo sac (fig. 17) and divides (fig. 18) to form two nuclei of which the micropylar moves toward the egg and may divide to form two to four nuclei; the chalazal one gives rise to a larger number, usually seven or eight (figs. 19–23).[1] Figure 23 represents a "plate" of eight free endosperm nuclei in an embryo sac which is cut transversely. The highest number of endosperm nuclei previously recorded in the family is four in *Cypripedium* (2).

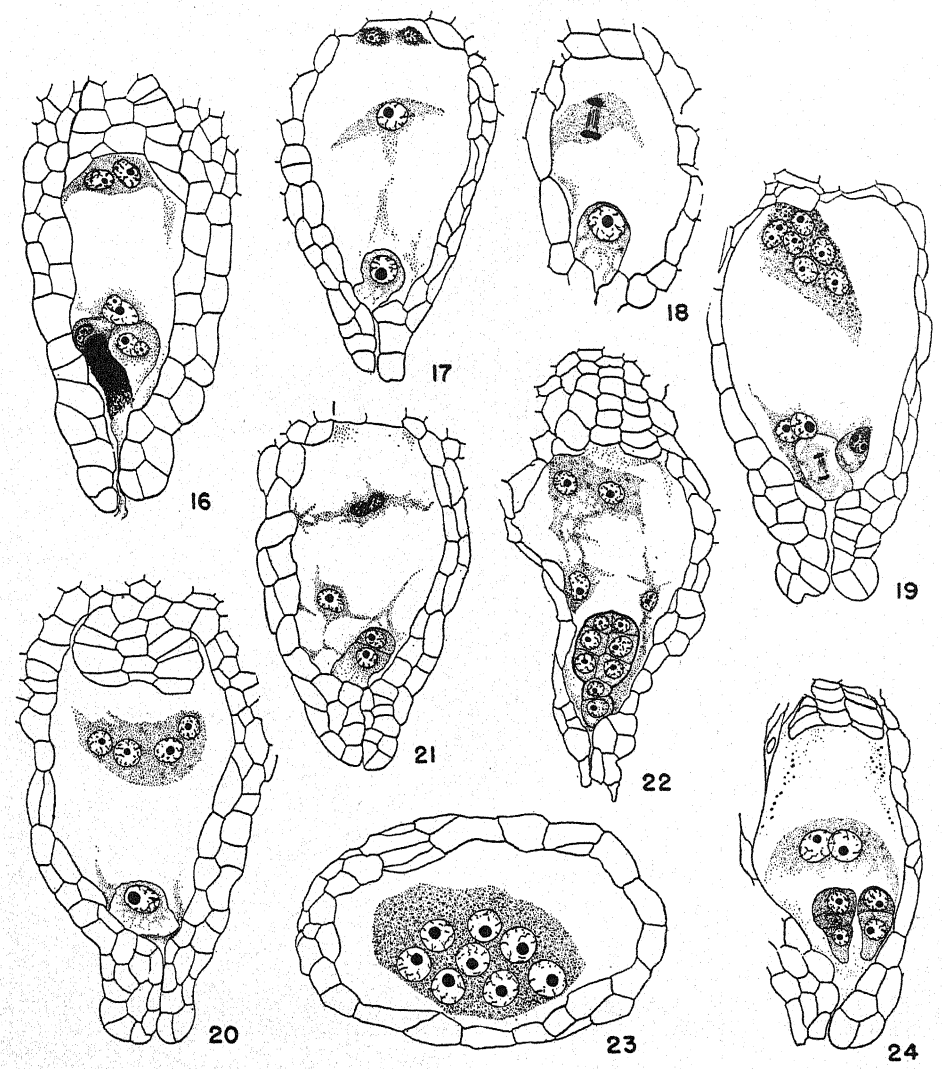
Normally the primary endosperm nucleus in *Vanilla* divides to form eight to ten free endosperm nuclei before the division of the zygote. In a few cases endosperm formation was arrested after the first or second division; figure 21 represents an instance in which even the

[1] All these figures are camera lucida drawings of single sections; only such endosperm nuclei as were visible in the particular section have been represented in a figure. However, about ten endosperm nuclei could be counted by examining the adjacent serial sections of these embryo sacs.

fusion of the two nuclei (upper polar nucleus and second sperm nucleus) is not complete, although the embryo has already attained the two-celled stage.

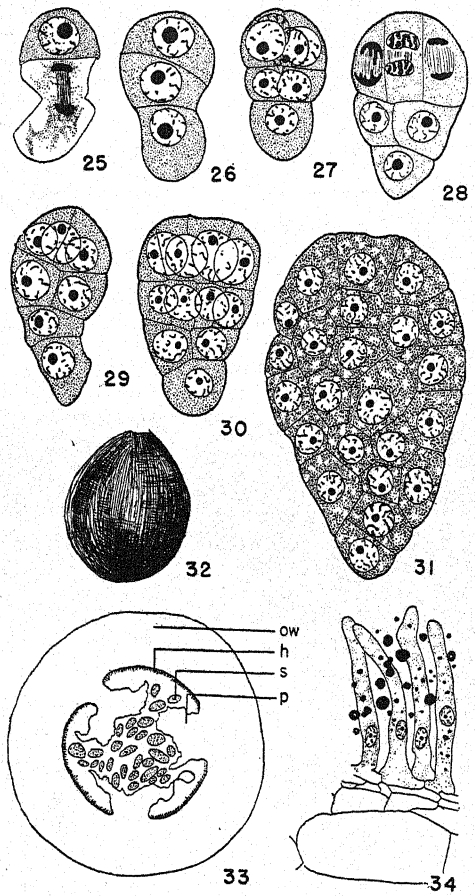
As in other orchids, the endosperm is soon used up by the growing embryo.

EMBRYO.—The first division of the zygote is transverse (fig. 21) and usually occurs after four or five endosperm nuclei are formed. By a further division of the micropylar cell (fig. 25) a proembryo of three cells is organized (fig. 26), as in the majority of orchids. The first two walls in the terminal cell are



FIGS. 16–24.—Fig. 16, double fertilization. ×600. Fig. 17, zygote, primary endosperm nucleus, and two antipodal nuclei. ×450. Fig. 18, first division of primary endosperm nucleus. ×450. Fig. 19, division of zygote; endosperm nuclei. ×450. Fig. 20, zygote and four endosperm nuclei, ×450. Fig. 21, two-celled proembryo and delayed fusion of second male nucleus with upper polar nucleus. ×450. Fig. 22, young embryo. ×450. Fig. 23, "plate" of eight endosperm nuclei (drawn from embryo sac cut transversely). ×600. Fig. 24, two proembryos lying side by side in embryo sac. ×450.

vertical and at right angles to each other, resulting in a group of four cells (fig. 27), each of which divides transversely (figs. 28, 30). Subsequent divisions are very irregular, resulting in an ovoid mass of cells (fig. 31). In embryos of mature seeds



FIGS. 25–34.—Fig. 25, basal cell of two-celled embryo in division. ×340. Fig. 26, three-celled proembryo. ×340. Figs. 27–31, subsequent stages in development of embryo. ×385. Fig. 32, mature seed; note opaque and smooth surface. ×105. Fig. 33, transverse section of fruit, showing placentae and development of unicellular secretory hairs from innermost layer of ovary. ×70. Fig. 34, secretory hairs enlarged. ×170.

there is always a well-marked depression at the chalazal end (figs. 39, 40).

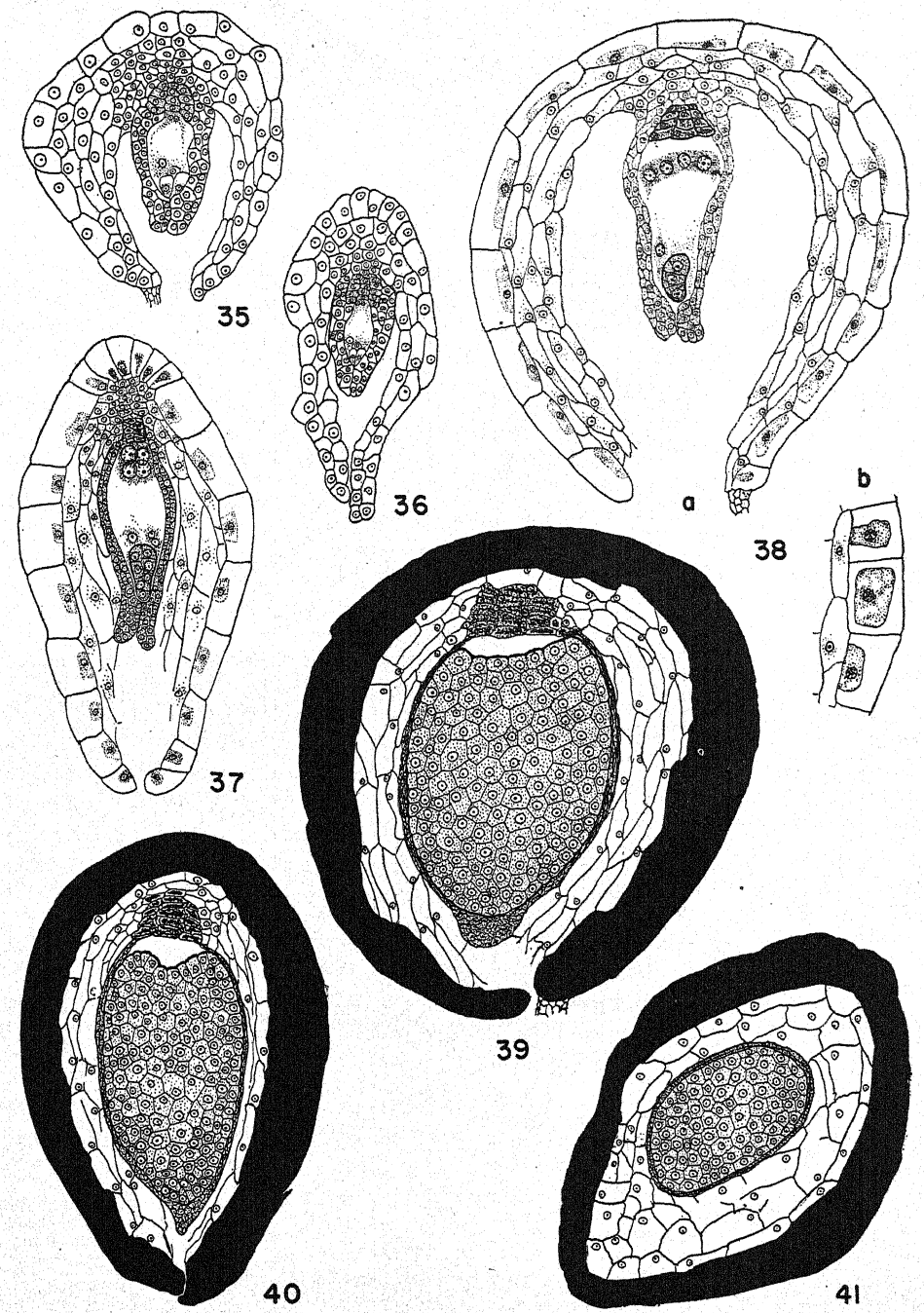
One ovule showed two embryos in the same embryo sac (fig. 24). Although the exact mode of origin of the additional embryo could not be determined, their position suggests that the three-celled embryo is the gametic one and that the two-celled embryo is derived from a synergid.

FRUIT AND SEED.—The fruit of *V. planifolia* is often described as a "bean" on account of its characteristic shape. It seems to be the only orchid possessing a fleshy fruit. RENDLE (3) has described it as dehiscing by two irregular longitudinal splits. Under cultivation, however, the pod does not show any split or rupture even when it is left on the plant for a long time and falls to the ground of its own accord.

After fertilization, the cells of the innermost layer of the ovary wall grow out into the cavity in the form of unicellular hairs which are packed with nutritive and other materials (figs. 33, 34) and are said to secrete the vanillin. They develop from the entire inner surface of the ovary wall, excepting the placentae.

In the majority of the orchids, because of the partial or complete disorganization of the cells of the inner integument, the embryo is suspended freely inside the seed coat, which is formed only from the outermost layer of the outer integument. In *V. planifolia*, however, the situation is different. Here persist not only the inner integument but also some of the original tissue of the nucellus lying at the chalazal end. These cells, which are small and very compactly arranged, stand out in sharp contrast to the large cells of the outer integument (figs. 35–40).

The epidermis of the seed coat (i.e., the outermost layer of the outer integument) undergoes important changes during the development of the embryo. The outer and lateral walls of these cells thicken enormously (figs. 37–38) until the cell cavity may be completely ob-



FIGS. 35–41. Figs. 35, 36, median longitudinal sections of young ovule (at about time of fertilization) in two planes at right angles to each other. ×42. Figs. 37, 38*a*, same as above but at later stage with young embryo and endosperm. ×42. Fig. 38*b*, three cells of outermost layer of outer integument enlarged to show gradual obliteration of cell lumen. ×270. Figs. 39, 40, sections of mature seeds in two longitudinal planes at right angles to each other. Note persistence of all cell layers of outer integument, crushed inner integument, cup-shaped depression at chalazal end of embryo, and sclerotic and opaque layer of seed coat. ×42. Fig. 41, transverse section of mature seed. ×42.

literated. Finally, a brownish-black deposit appears in the cells and intensifies to such an extent that the entire layer becomes opaque (figs. 39–41). It is smooth and highly sclerotic, without any recognizable reticulations or pattern (fig. 32), and thus offers a complete contrast to the condition in other orchids, which have a reticulate and transparent seed coat. The only cases similar to *Vanilla* are *Apostasia*, *Adactylus*, and *Selenipedium* (1).

Summary

1. In the orchid *Vanilla planifolia* the microspores do not remain united to form compound grains, massulae, or pollinia, but lie free inside the anther. The pollen grains are shed at the two-celled stage.

2. The nucellus shows a greater development than in most other orchids, and the nucellar cells at the chalazal end persist even in the mature seed.

3. The outer integument consists of four to six layers of cells which persist in the seed. In other orchids only the outermost layer is recognizable in the seed.

4. The inner integument is also two- to three-layered and, although flattened and crushed, remains distinguishable for a long time.

5. The embryo sac arises from the chalazal cell of a row of three cells, consisting of a micropylar undivided dyad cell and two megaspores. After the four-nucleate stage, the two chalazal nuclei do not divide further, resulting in a six-nucleate embryo sac.

6. Double fertilization takes place normally, but, since there is no lower polar nucleus, the primary endosperm nucleus is diploid and not triploid.

7. As many as eight to twelve endosperm nuclei were counted in certain ovules, which is noteworthy in view of the fact that ordinarily endosperm formation is completely suppressed in the orchids.

8. One case of twin embryos was noted. One of them was gametic in origin and the other probably arose from a synergid. The embryo of the mature seed shows a terminal depression.

9. Both integuments take part in the formation of the seed coat. The outermost layer of the outer integument becomes highly sclerotic with black deposits and is opaque and smooth. This is in marked contrast to the condition in most other orchids, where there is a transparent seed coat with a reticulate surface formed only by the outermost layer of the outer integument.

I am indebted to DR. P. MAHESHWARI for revising the manuscript.

BIOLOGICAL LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

1. NETOLITZKY, F. Anatomie der Angiospermen-Samen. Berlin. 1926.
2. PACE, L. Fertilization in *Cypripedium*. BOT. GAZ. 44:353–374. 1907.
3. RENDLE, A. B. Classification of flowering plants: monocotyledons. Cambridge University Press, London, 1930.
4. SWAMY, B. G. L. Self-pollination in two orchids. (In press.)

CURRENT LITERATURE

Luther Burbank: A Victim of Hero Worship. By WALTER L. HOWARD. (*Chronica Botanica* 9 [5/6]: 299–522. 1945–1946.) Waltham, Mass.: Chronica Botanica Co., 1946. $3.75.

The flow of comments and writings concerning LUTHER BURBANK and his work has notably slackened during the last decade. With the lapse of time since his death in 1926, what is said about him and his work has become more objective and less prejudiced either for or against him or his efforts. The recent volume by Dr. W. L. HOWARD states its purpose in a portion of its prologue: "With none too gentle hands I have endeavored to tear away these hindering habiliments to see what the real BURBANK looked like. And behold, a pristine figure emerges that is every inch a man of worth, a man of original ideas; a man with a definite mission in life, fully capable of standing on his own feet as a lone worker in the field of science." The author states further that he is "convinced that BURBANK possessed the talent and attributes of a conventional scientist and [that] there would have been no question about his acceptance had he been taken in hand when young and given the proper training demanded by the code. . . . . For [his] concrete accomplishments and because he was the ferment that stirred others to advance the science of breeding, an intangible achievement, to be sure, but his greatest from the viewpoint of science, he is definitely entitled to a place in the hall of fame; but, all factors duly considered, the best that I can do is to assign him a front seat among the minor prophets, a front seat because of the unusual fact that here is one prophet, at least, who was not without honor among his home people."

Dr. HOWARD has based his book on his personal contact with BURBANK, conversations with many individuals who knew him intimately, BURBANK's own writings, and a firsthand opportunity to observe and evaluate many of BURBANK's horticultural creations. Although many of the latter have proved to have very little permanent value, a number of others, including the Burbank potato and several varieties of plums, have proved of great commercial importance.

When one reads of the vigor and persistence with which BURBANK was accustomed to work and of the myriad interruptions of his time and efforts by the interested or by the merely curious, he marvels not at his occasional manifestation of irritability but at his infinite patience, tolerance, and kindliness. For him to have completely resisted the adulations showered upon him, or to have withstood the flattery from others for their own personal gain, would have marked BURBANK as superhuman. That he quite obviously was not.

Dr. HOWARD's book is deserving of reading and study as an objective evaluation of BURBANK and his work. It is of equal interest in its disclosure of the author's own mind and opinions. Coming as his remarks do, from one who has had many years of experience with men and problems in the applied and scientific phases of horticulture and agriculture, one who has had broad administrative contacts in dealing with the acts and minds of men, his comments have much more than passing interest. Much of what he has said about BURBANK and about science and its workers has needed saying for some time. Those fortunate enough to have an opportunity to read this book, especially younger scientists, will wish to keep it near at hand so that they may reread it often for its stimulating pleasure, for its clarity, and for the pointedness of its comments.—E. J. KRAUS.

Garden Lilies. By ALAN and ESTHER MACNEIL. New York: Oxford University Press, 1946. Pp. xiv+226. $3.50.

This is a rather small handbook which has been written by experienced growers and lovers of lilies for the gardener who wants to grow these plants successfully. The book is arranged in two parts.

Part I considers such topics as the selection of suitable varieties, sites for planting them in order to bring out the best effects from size of plant and type and color of flower. Practical information on planting, on culture and fertilization, and on propagation follow. Next is a chapter dealing with lilies for pots and exhibition, with suggestions regarding size of pot and bulb, soil, moisture, and temperature requirements, and a list of varieties adapted for pot culture with brief recommendations for preparing lilies for exhibition. Natural hazards and pests are listed, with suggestions for their control or amelioration. A discussion of lily diseases and their control forms a valuable and concise practical summary of the present knowledge in this area. This part is concluded with an interesting and informative discussion of lilies through the ages.

Part II is devoted largely to a descriptive list of lilies. Although the descriptions are designed not for the botanist but for the gardener, they are nevertheless accurate, concise, and generally adequate. The list comprises nearly half the book and is a valuable feature, since it furnishes much worth-while information for all who may be interested in this lovely garden plant. A selected list of references precedes the index.—J. M. BEAL.

Forest Soils and Forest Growth. By S. A. WILDE. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert & Co., 1946. Pp. 241. Illus. $5.00.

The appearance of this book fulfils an urgent need among ecologists and foresters, especially those engaged in land management, reforestation, nursery practice, and soil conservation.

A historical and introductory chapter is followed by three chapters on genesis of soils and by one on the biological structure of the forest cover and its relation to environment. There are separate chapters on physical and chemical properties of forest soils, their organisms, forest humus, soil-forest types, forest soil survey, and soils and tree-planting. Practical applications of knowledge of forest soils are brought out in chapters on amelioration of forest soils, silvicultural cuttings in relation to soils, and productivity of forest soil and forest management. The final five chapters deal with various problems in establishment and management of forest nurseries, with special emphasis on various aspects of fertilization, watering, and control of parasitic organisms. Here WILDE contributes a very large part from his own exhaustive researches conducted over a period of several decades. The book is well illustrated with thirty-eight figures (maps, charts, and graphs) and seven plates, besides a number of clearly done sketches that lend interest and a decorative motif.

The author has rather completely reviewed the literature. His firsthand knowledge of Russian and German, besides a half-dozen other languages, makes this an unusually valuable contribution.

By training, Dr. WILDE is a forester as well as a pedologist. This rare combination gives the book a balanced view of the interdependence of silviculture, ecology, and soil science that is largely lacking in existing textbooks which deal with the topic either of silviculture or of soils but fail to integrate the two subjects properly. This valuable book is thus a "must" for every ecologist and forester. It is one of the most useful of references.—J. H. STOECKELER.

Crop Production and Environment. By R. O. WHYTE. London: Faber & Faber, Ltd., 1946. Pp. 372. Illustrated. 12*s*.

Under this general title one might reasonably expect a discussion of any aspect of applied plant science, but, obviously, one book could not cover all aspects of crop production and environment. On the basis that the physiological processes leading to growth and reproductive development are fundamental to crop production, WHYTE deals with the literature related to the initiation and development of flowers in plants as influenced by the decisive environmental factors, light and temperature.

The book is not simply a review but is a very reasonable attempt to evaluate and to correlate the literature in the fields of photoperiodism, vernalization, and phasic development. The author has selected certain critical investigations for detailed consideration and has included many excellent illustrations taken from the original papers. An extensive review of the factual data is presented, and considerable space is devoted to the various hypotheses which have been developed in relation to some of the facts. This latter inclusion is fortunate, since the obvious inadequacy of any one of the hypotheses in relation to all the available facts demonstrates the need for more critical experimentation. The similarity among some of the more recent generalizations encourages one to hope that soon additional generalizations will serve to correlate the flowering responses of most or even of all plants with changing environmental conditions.

WHYTE uses the terms "growth" and "development" in a restricted sense and defines them as follows: "Growth is the accumulation of dry matter or increase in size of a plant, development of progress towards reproduction by externally recognizable or invisible stages." The book is primarily concerned with the problems of development.

The Introduction gives an outline of the scope of the book, and the first chapter includes a historical review of the development of the studies on photoperiodism and vernalization, with some detail of the early work of KLEBS, GASSNER, and GARNER and ALLARD. There follows a chapter on the concept of phasic development, giving a concise statement of LYSENKO's work and hypothesis, together with the modifications introduced by subsequent workers. In the remaining chapters the influences of darkness, light, and temperature are discussed in relation to photoperiodism and phasic development. Extensive reference is made to the Russian literature. Most of the hypotheses which have been developed in connection with this work receive considerable attention. One brief chapter is devoted to the problem of breeding plants in relation to phasic analysis—a welcome presentation of a controversial subject. The last four chapters discuss the responses of economic crops and possible practical application of our knowledge of developmental physiology.

This book should prove to be valuable to those interested in the physiological processes leading to flowering in plants, and it should be especially invaluable to the research worker. Perhaps its greatest contribution will be in its stimulation of new research, since it summarizes the present status of our information in this field and points the way for new investigations to fill in the gaps in our knowledge.—K. C. HAMNER.

ANATOMY OF WASHINGTON NAVEL ORANGE RIND IN RELATION TO WATER SPOT

FLORA MURRAY SCOTT AND KATHARINE C. BAKER

Introduction

In southern California the damage caused by water spot to the crop of Washington navel oranges is a cause of increasing concern to the citrus industry (10, 11, 13, 20, 22, 24, 41).

The first visible symptom of injury is a local swelling and waterlogging of the rind in the maturing fruit. The air normally present in the respiratory chamber beneath the guard cells, and also in the intercellular spaces, is replaced by fluid, and, as a result, the surface of the rind becomes raised and more or less translucent. The injury occurs during cold rainy periods in winter, and the progress of the disease appears to depend on weather conditions. If a dry spell follows a rain, the rind swellings may recede within a few days and the fruit remain normal. If, however, the damage has gone too far, the affected areas become sunken, dried out, and brown in color, marking the fruits as culls from the grower's standpoint. If the rain continues, the initial swelling may spread, and, in a matter of hours or days, the epidermis may crack in all directions. Again the weather determines the fate of the fruit. Should the rain cease, the cracked epidermis will heal by normal cicatrization. The rind at maturity will be scarred and shriveled, but the fruit will be otherwise undamaged. On the other hand, if wet weather persists, the ubiquitous spores of molds such as *Penicillium* and *Cladosporium* may enter the cracks, germinate, and spread through and rot the entire fruit within 48 hours.

The localized swelling of the rind tissue in water spot may be classified anatomically as intumescence (26). The behavior of the tissue in *Citrus*, however, differs markedly from that in the intumescences induced in poplar by stimuli as diverse as stagnant moist air and hormone treatment. In poplar the hypertrophy of certain individual cells is exaggerated, whereas in the flavedo cells of *Citrus* the expansion of individual cells is barely noticeable microscopically.

Among the numerous publications on *Citrus*, comparatively few are concerned with the detailed structure of the rind (2, 15, 39, 42). The fruit of *Citrus*, the characteristic hesperidium, consists of carpels, morphologically leaves. The structure of the typical leaf, with its epidermal, photosynthetic, and conducting tissues, is paralleled in the outer epidermis, the flavedo and albedo, and in the vascular network of the fruit. The endocarp or pulp, consisting of juice sacs, is peculiar to the fruit. Oil glands are numerous near the surface in the fruit as in the leaf.

At the present time plant pathologists and physiologists are engaged in the investigation of the causes and possible methods of control of water-spot injury (6). The present paper deals with the anatomy of the rind of the ripening fruit and forms a part of the background necessary for the interpretation of current experimental work.

Material and methods

Material was collected from the university experimental orchards in Los

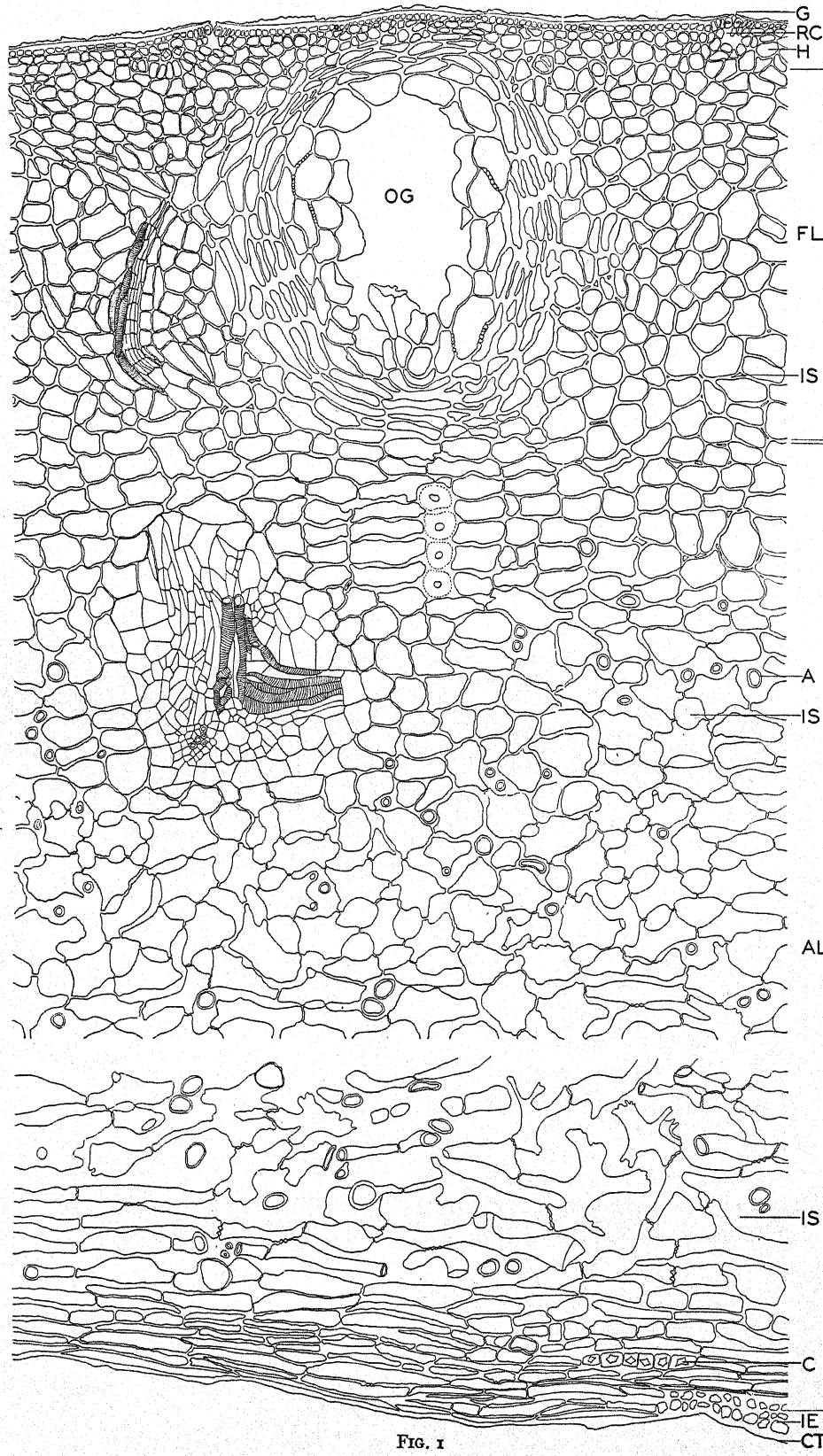


FIG. 1

(Legend on facing page)

Angeles and Riverside and also from the Foothill Ranch, Azusa.[1] The bulk of the fruit had been oil-sprayed at various times of the year in accordance with usual orchard practice. In all groves, however, a number of trees were left unsprayed, so that untreated fruit was available for comparison. In addition, in order to determine the effects, if any, of oil treatment on the anatomy of the rind, growing and picked fruits were dipped in concentrated spray oil.

Fruits in all stages of growth were sectioned transversely, radially, and tangentially. They were examined immediately, and after treatment with the standard microchemical reagents for ergastic substances and wall materials (40). Hesperidin, which crystallizes immediately on dehydration, is present in fruits of all ages and is particularly abundant in the younger.

Plasmodesmatal connections are apparent on application of IKI followed by H_2SO_4 (5%, or stronger if irrigation is used). The results of this method are confirmed by the standard technique of CRAFTS (7).

Wax and cutin are distinguished clearly under the polarizing microscope and also in sections stained with Sudan III or Biebrich Scarlet. In addition to the latter reagents, H_2SO_4 ($\pm$75%) is useful in the study of the cutinization of the respiratory chamber and of the tissues involved in frost injury and wound healing. The radial canals of the outer walls of the epidermal cells appear most clearly after irrigation with IKI with or without the addition of H_2SO_4 ($\pm$5%).

[1] The writers are indebted to Mr. PAUL MOORE for his assistance in experimental treatment of fruit in the field.

In the observation of the vacuoles of living protoplasts, neutral red and methylene blue are effective (18). The latter, because of the more marked color contrast, proved the more useful, but both stains give the same qualitative results.

In the study of albedo development thick sections are essential in determining the main structural pattern of cells and intercellular spaces. Macerated material is necessary in the observation of individual cells.

Observations

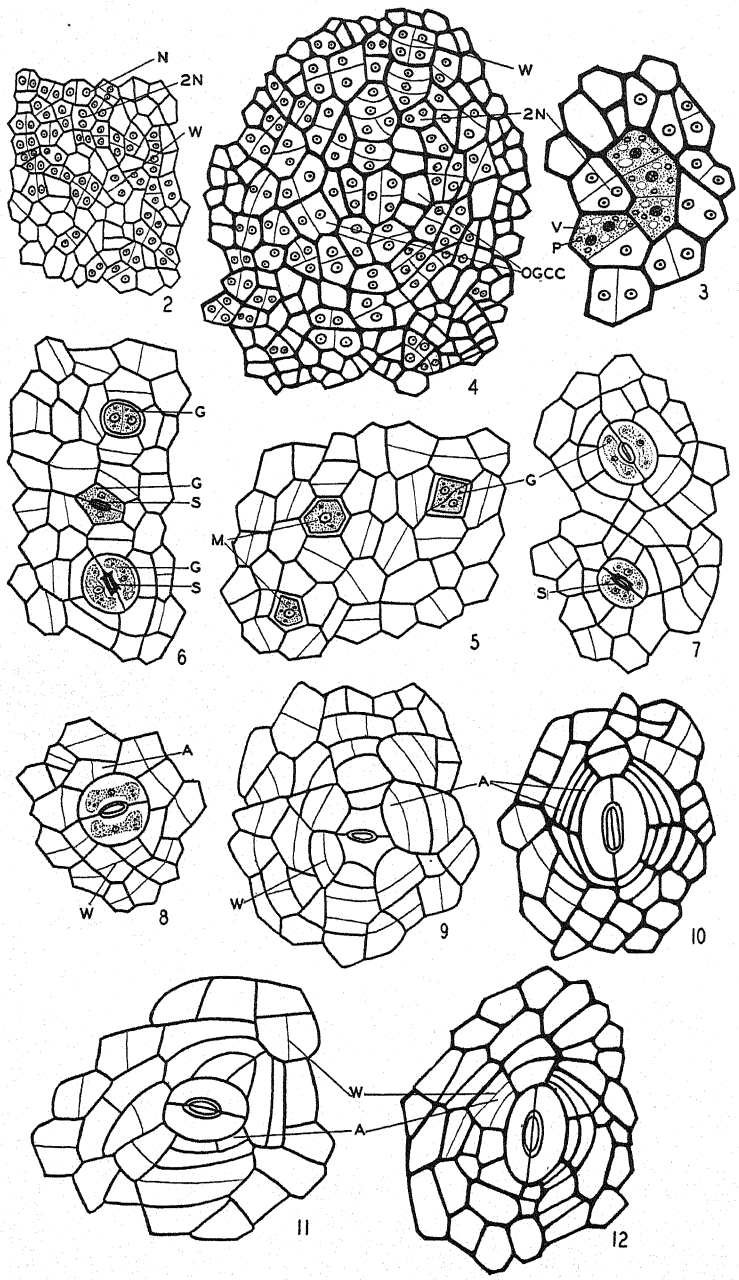
The peel or pericarp, consisting of epicarp, hypodermis, and mesocarp (42), includes the following tissues: epidermis, hypodermis, flavedo, oil glands, vascular bundles, albedo, and inner epidermis (fig. 1). A typical Washington navel pericarp is 8 mm. in thickness; variations from this occur in the albedo tissue.

EPIDERMIS

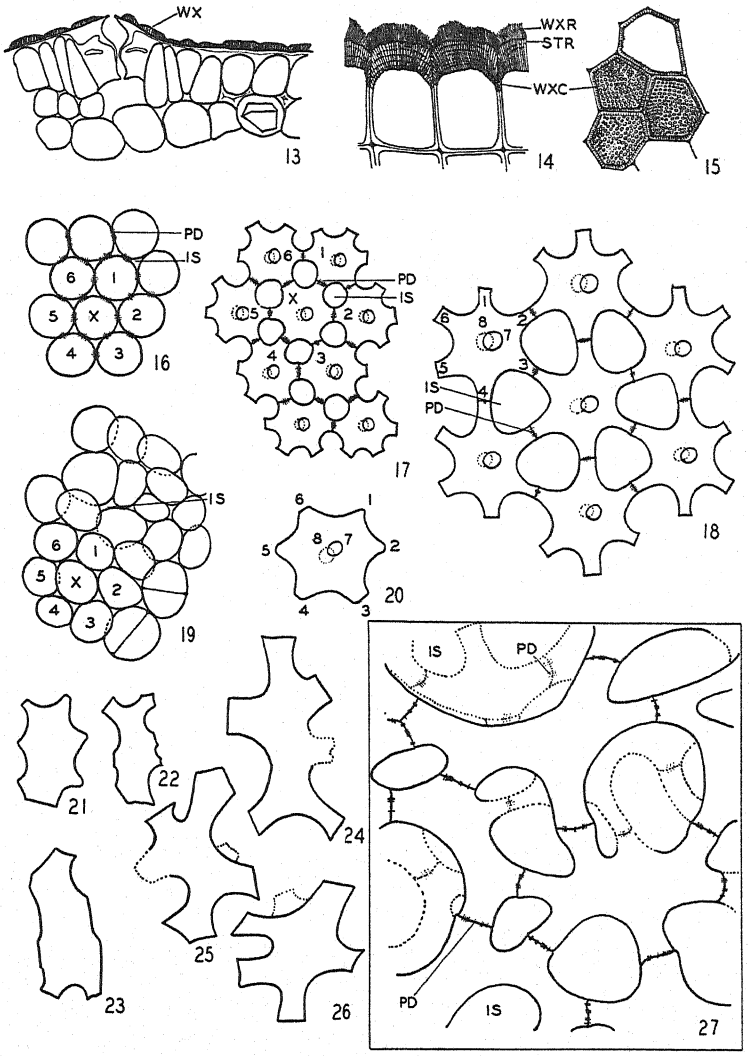
In the epidermis four types of cells may be distinguished: epidermal, guard cells, accessory cells, and oil-gland cover cells (figs. 2–12). Epidermal cells are polygonal in outline and roughly isodiametric. The outer wall, consisting of cellulose and pectic substances, horizontally stratified, is cutinized and further protected by a layer of wax (figs. 13–15). Within the living cells may be identified cytoplasm, nucleus, one or more vacuoles, and plastids. The plastids are approximately equal in size to those of the flavedo, but they are relatively few in number (fig. 50).

Cell division may occur in any region of the epidermis, in the equatorial, stem,

FIG. 1.—Transection of rind of mature fruit 8 mm. from outer to inner epidermis; approximately 4 mm. of mid-albedo has been omitted: *G*, guard cell; *RC*, respiratory chamber; *H*, hypodermis; *FL*, flavedo; *A*, arm of albedo cell; *IS*, intercellular space; *AL*, albedo; *C*, calcium oxalate crystal; *IE*, inner epidermis; *CT*, cutin; *OG*, oil gland.



FIGS. 2–12.—Figs. 2, 3, epidermis, maturing fruit, surface view; cell division, binucleate cells and recently formed thin walls: *N*, nucleus; *2N*, binucleate cell; *W*, new wall; *P*, plastid; *V*, vacuole. Fig. 4, epidermis, maturing fruit, surface view; cell division in oil-gland cover cells: *OGCC*, oil-gland cover cells. Figs. 5–7, epidermis, young fruit, surface view; differentiation of guard cells from mother cell: *G*, guard cell; *M*, mother cell; *S*, stoma. Figs. 8–12, differentiation of accessory cells: *A*, accessory cells; *W*, new wall. Overhang of young accessory cells (fig. 9) is common.



FIGS. 13–27.—Figs. 13–15, epidermis, maturing fruit, transections and surface view: *WX*, wax scale; *WXC*, wax canal; *WXR*, wax rodlet; *STR*, stratification. Figs. 16–18, diagrams, differentiation of eight-armed cells in mid-albedo; a typical cell, X, surrounded by eight adjacent cells, arms 7 and 8 at right angles to plane of paper: *PD*, plasmodesmata; *IS*, intercellular space. Fig. 19, mid-albedo of young fruit, transection, camera lucida (compare with fig. 16). Fig. 20, mid-albedo, macerated cell (compare with fig. 18). Figs. 21–23, outer albedo, macerated cells (compare with figs. 28, 29). Figs. 24–26, mid-albedo, macerated cells (compare with figs. 18, 27, 33). Fig. 27, mid-albedo, transection, camera lucida.

or navel zones of the maturing fruit. In fruits considered picking-ripe, binucleate cells lie adjacent to others both older and younger. In the latter the newly formed cell walls may still be barely visible (figs. 2–4). The areas of active cell division are sporadic, patches, streaks, or disconnected networks. They may radiate in any direction from the periphery of large or small oil glands, or may wander between the complex of stomata in the interglandular areas. Since stretching growth necessarily occurs before and after cell division, the comparatively weak external walls of these meristematic cells represent areas of potential cracking.

GUARD CELLS.—The paired guard cells of the stomatal apparatus arise from a mother cell, which at first, in surface view, is distinguished from the surrounding cells only by wall thickening, density of cell content, and accumulation of oil (figs. 5–12). This parent cell is four- to six-sided and is surrounded by the same number of cells of approximately equal size. When cell division occurs, the position of the stoma is soon defined by localized thickenings along the central one-third of the median walls. As the guard cells expand, the stoma widens and becomes elliptical in outline and clearly defined by the cutinized rim (figs. 5–9). In radial section the young guard cells are seen to possess the normal covering of cutin on the outer epidermal surface and on the wall of the stomatal pore (fig. 36). In older stomata cutinization may extend across the inner tangential wall and thence over the surfaces of the cells which line the respiratory chamber (figs. 37–40). By treatment with H_2SO_4, the surface cuticle and the cutinized saclike lining of the respiratory chamber may be isolated. The thickness of the latter ($\pm 2\ \mu$) is less than that of the cuticle of the outer surface ($\pm 3\ \mu$).

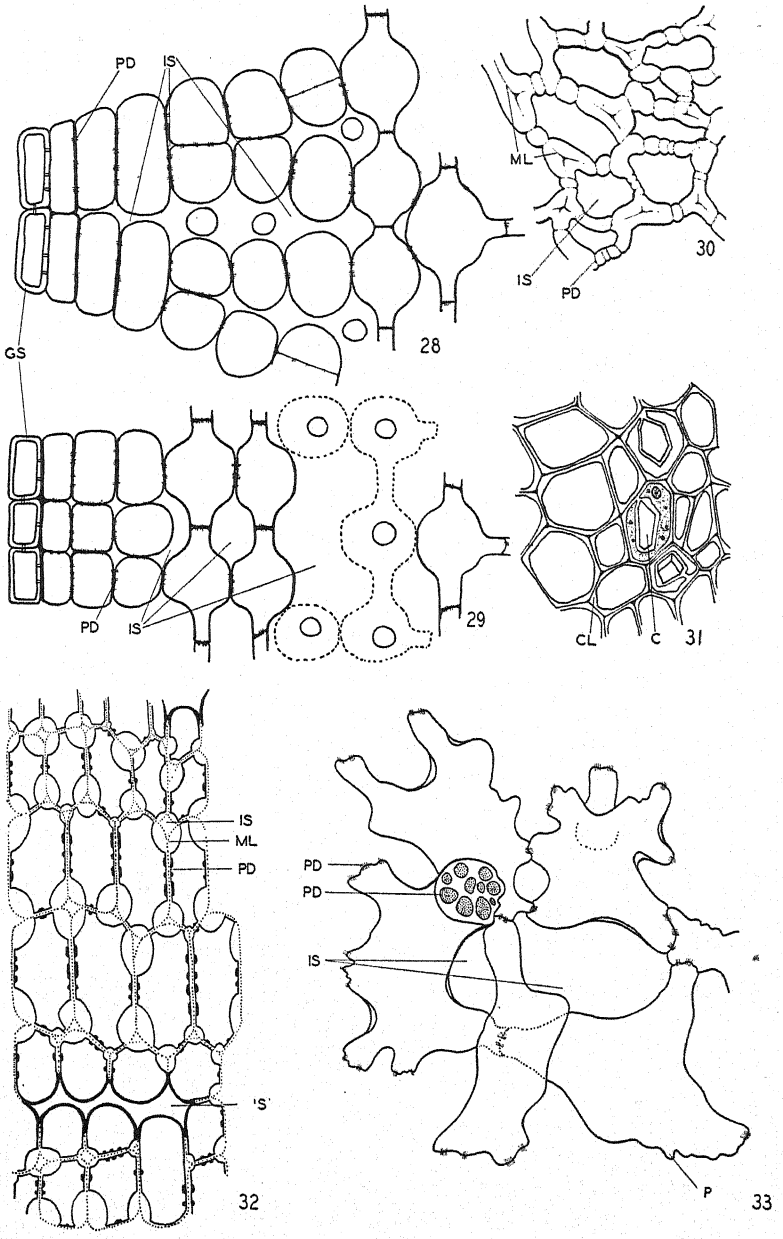
ACCESSORY CELLS.—As the guard cells differentiate and mature, the adjacent cells divide by radial and tangential walls (figs. 5–12). The divisions may be somewhat erratic, but in a typical stomatal area the guard cells are surrounded by from three to four rows of narrow accessory cells. These may extend along six-eighths or seven-eighths of the outer rim of the guard cells while the remaining arc or arcs are occupied by undivided polygonal elements.

In fruits of all ages the surface of the subsidiary cells is comparatively weakly cutinized (figs. 36–39). This may be observed in radial section but is strikingly demonstrated when tangential sections of the epidermis are treated with H_2SO_4 ($\pm 80\%$) (figs. 48, 49). On penetration of the reagent the thinly cutinized walls of the accessory cells immediately swell and thereafter completely disappear, while the heavy cuticle of the isolated guard cells and the rest of the epidermis remains intact.

OIL-GLAND COVER CELLS.—The area of the oil-gland cover cells is proportional to the diameter of the underlying gland (figs. 1, 4). In the mature fruit the cells are somewhat flattened and in surface view polygonal. They may be smaller or larger than the adjacent epidermal cells, since cell diameter varies with the age of the gland. Oil droplets are generally present in the cells, the protoplast of which is further distinguished in tangential section by its comparative translucence.

NUMBER AND DISTRIBUTION OF STOMATA AND OIL GLANDS.—In a recent paper the number and distribution of oil glands and of stomata in the mature fruit have been statistically treated (41).

The repetitive unit in the complex pattern of the rind is the individual oil gland with its orbit of satellite stomata. In a fruit 0.5 cm. in diameter, a typical



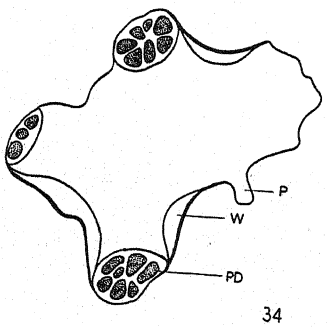
FIGS. 28–33.—Figs. 28, 29, diagrams, outer albedo, transection and radial section; differentiation of branched cells (compare with figs. 16–18, 32): *GS*, gland-sheath cell. Fig. 30, mid-albedo, young fruit, transection; plasmodesmata: *ML*, middle lamella. Fig. 31, hypodermis, maturing fruit, surface view: *C*, calcium oxalate crystal; *CL*, collenchymatous thickening. Fig. 32, outer albedo, developing fruit, transection, camera lucida; early formation of arms and intercellular spaces. Fig. 33, mid-albedo, mature fruit, transection; intercellular spaces, arm and wall plasmodesmata: *P*, papilla.

gland is surrounded by a single orbit in which the stomata, five to eight in number, are approximately equidistant from the gland and from each other. As the gland and, concurrently, the interglandular ridge increase in volume, a second orbit of stomata differentiates. Meantime during the expansion of the fruit, additional oil glands are initiated, each with its potential complement of stomata. Sooner or later, therefore, adjacent orbits intersect, and, as a result, in any interglandular area of the epidermis, stomata of different ages and in varying degrees of development can be identified. The differentiation of stomata is most active in the youngest fruits. It is estimated that, in fruits 1 cm. in diameter, half of the total number of stomata present in the mature fruit are already in process of development. In fruits ± 2.5 cm. in diameter, which are approximately one-third grown, differentiation still continues, and young stages of stomatal development are still of common occurrence. In mature fruits, on the other hand, while cell division persists as already mentioned, all stomata are practically mature.

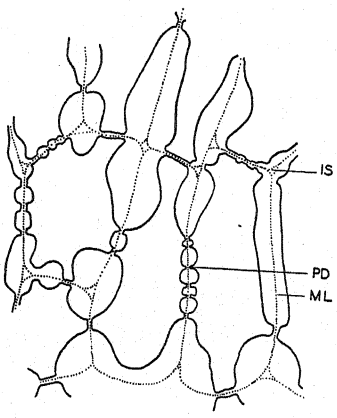
That cell size increases with age in epidermal and other tissues is shown in table 1.

WAX.—The slight but definite dulness of surface due to the coating of wax is more readily observed on unsprayed than on sprayed fruit. At the time when the groves are normally sprayed, the fruits are approximately two-thirds grown. Oil-spraying does not inhibit the subsequent secretion of wax on the still expanding fruit.

The wax coating consists of a layer of flattish scales roughly equal in area to the varying surfaces of the epidermal cells of all types, including the specialized guard, accessory, and oil-gland cover cells (figs. 13–15). On closer examination each wax platelet is seen to consist of minute vertical rodlets less than 1 μ in diameter and 2–4 μ in length (8, 9, 29). In radial section very fine canals are evident in the outer walls of the epidermal cells, the openings of which, when seen in surface view, appear as minute pits. The wax which is synthesized within the epidermal protoplasts, after exudation through the channels, solidifies to form the closely packed and coherent surface



34



35

FIGS. 34–35.—Fig. 34, mid-albedo, mature fruit, individual cells: *W*, curving walls. Fig. 35, outer albedo, developing fruit, transection; early arm and intercellular space formation.

rodlets. The radial canals may also allow of the intermittent transport of the cutinogenic fatty acids, the deposition of which accounts for the increasing thickness of the cuticle.

The thickness of the wax coating in the maturing fruit varies somewhat as might be expected. Buckling and overlapping of scales is doubtless due to the persistence of cell division and consequent expansion of the epidermis. A curious heaping-up of wax platelets is commonly seen in fruits stained overnight in methylene blue. On such fruits there may appear dark-blue, somewhat irregular longitudinal bands, ±3 mm. in width, which may run the entire length of the fruit from stem to navel. Along such streaks the wax scales may be piled up erratically, two or more deep, and methylene blue heavily absorbed by them. The streaks are readily erased by light rubbing. The distribution of the bands bears no obvious relation to segment orientation, or to rind venation, and must therefore result from a basic pattern of growth in the epidermis as a whole.

TABLE 1

SIZE OF TYPICAL CELLS OF WASHINGTON NAVEL ORANGE FRUIT AT DIFFERENT STAGES OF GROWTH AND WALL THICKNESS IN MATURE FRUIT

TYPICAL CELL OF:	SIZE OF TYPICAL CELLS (μ)				THICKNESS OF WALLS (μ)
	Fruit 2 cm. diameter	Fruit 3 cm. diameter	Fruit 4 cm. diameter	Mature fruit 8 cm. diameter	Mature fruit 8 cm. diameter
Cuticle	1	1	1.5	3	.....
Epidermis	6×10	7×10	9×10	11×15	0.75
Hypodermis	8×10	12×12	13×15	18×23	1.50
Flavedo	15×19	21×24	23×24	40×50	1.50
Outer albedo	25×30	27×38	30×64	56×63	1.00
Inner albedo	27×34	33×60	30×150	34×200	1.00
Inner epidermis	15×55	15×75	15×75	15×20–15×100	.....
Epidermis over oil gland	.....	.....	.....	10×10	0.75
Flavedo over oil gland	.....	.....	.....	15×25	0.75–1.50
First layer of oil gland, median section	42×75	45×75	45×75	86×182	1.00
Second layer of oil gland	10×60	25×65	30×75	50×100	1.50
Third layer of oil gland	12×67	12×67	25×75	28×100	2.00–8.00
Spiral vessel	.....	.....	.....	8×300	.....

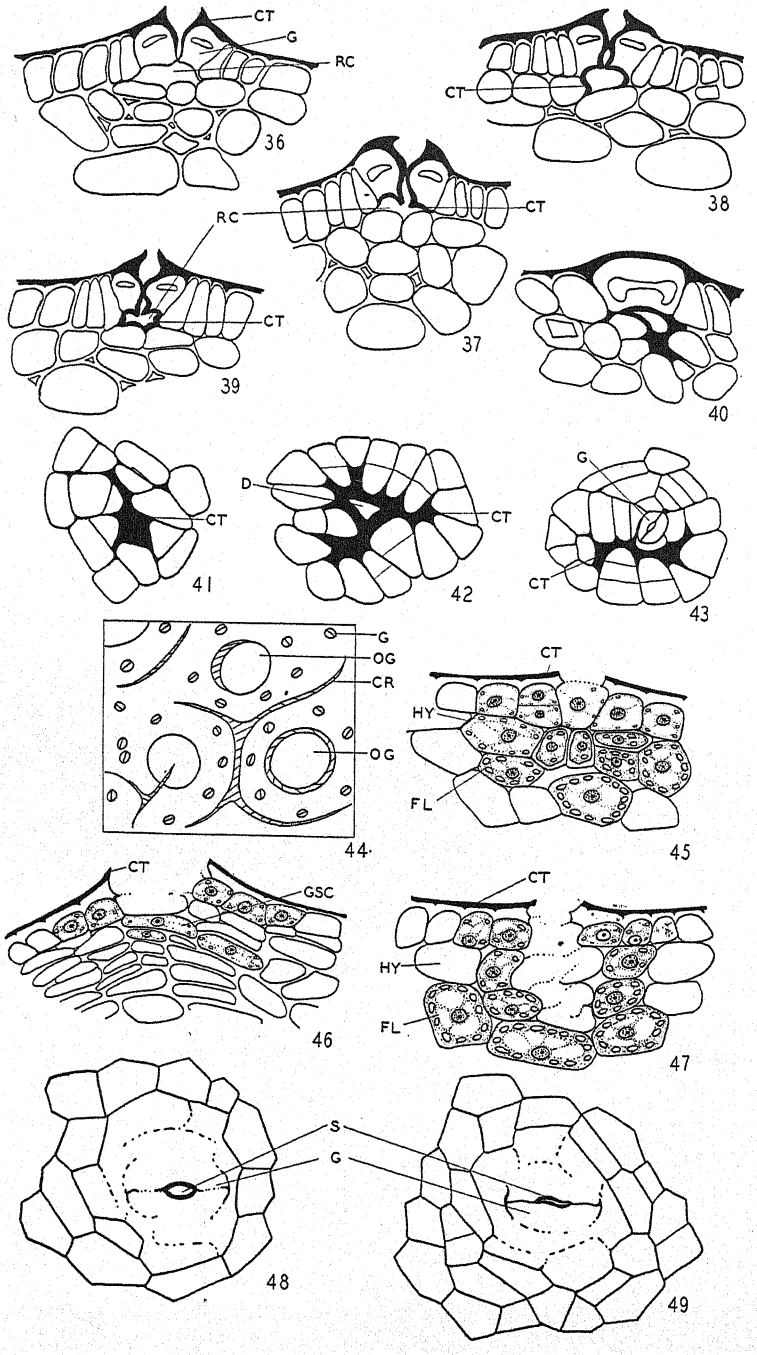
HYPODERMIS

Beneath the epidermis lies the hypodermis, a somewhat ill-defined layer varying from one to three cells in thickness. It consists of polygonal or ellipsoidal cells with characteristic collenchymatous wall thickenings. Intercellular spaces, although small, are present throughout this tissue in the mature fruit (figs. 1, 13).

Crystal idioblasts are extremely abundant, ranging in number from 350 to 900/sq. mm. (fig. 31). As in the leaf, they encircle, at a distance, both stomatal chambers and oil glands. The earliest stages of development in the youngest fruits are difficult to observe in fresh material owing to the abundance of hesperidin.

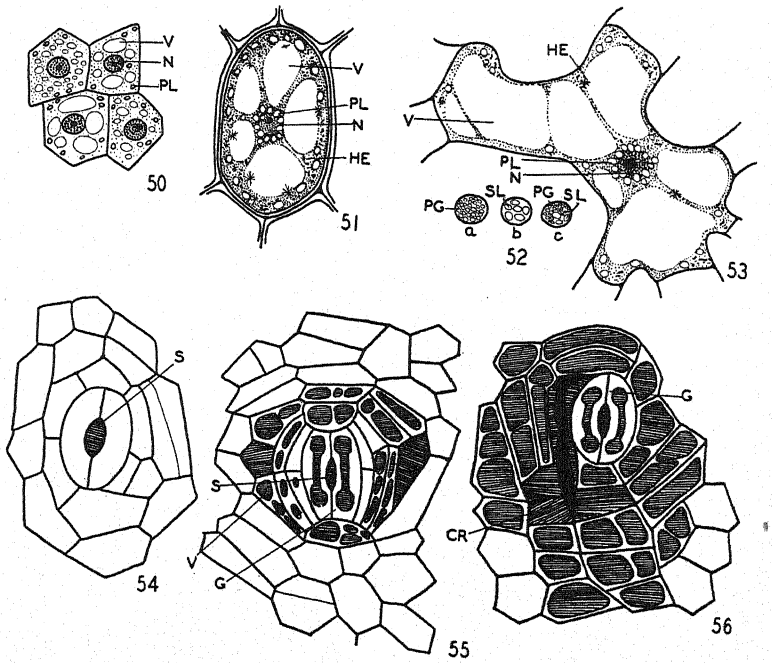
FLAVEDO

The flavedo is the zone of plastids, oil glands, and vein endings (fig. 1). As in



FIGS. 36–49.—Figs. 36–40, progressive cutinization of respiratory chamber: *CT*, cutin; *G*, guard cell; *RC*, respiratory chamber. Figs. 41–43, epidermis, young fruit, surface view; healing of incipient cracks by cutin: *D*, dead cell. Fig. 44, epidermis, surface view, diagram; pattern of early major cracks: *CR*, crack; *OG*, oil gland. Figs. 45–47, outer rind, transections; progressive cracking: *GSC*, gland-sheath cell; *HY*, hypodermis; *FL*, flavedo. Figs. 48, 49, epidermis, surface view; dissolution of accessory cells in H_2SO_4.

epidermis and hypodermis, cell division continues in the outer flavedo until the fruit is approximately full grown. The cells are polygonal in outline with comparatively small intercellular spaces. Increase in volume of the inner flavedo results from expansion both of cells and of intercellular spaces. The lobed cells of vary. Chromoplasts contain numerous minute granules of pigment, less than 1 μ in diameter. Typical leucoplasts may inclose a varying number of starchlike grains, 1–3 μ in diameter, which stain faintly blue with IKI. Certain other plastids, intermediate in ergastic content, contain both pigment and starch.



FIGS. 50–56.—Fig. 50, epidermal cells, surface view; protoplast: *V*, vacuole; *N*, nucleus; *PL*, plastid. Fig. 51, flavedo cell; protoplast: *HE*, hesperidin. Fig. 52*A*, *B*, *C*, plastids with granules: *PG*, pigment; *SL*, starchlike granules. Fig. 53, albedo cell; protoplast. Figs. 54–56, epidermis, surface view; staining by methylene blue of vacuoles and stomatal plugs: *CR*, crack.

this zone form a transition to the characteristic branched elements of the albedo. The cells adjacent to the oil glands in the outer and inner flavedo are thick-walled, flattened, and characteristically pitted.

Plastids (figs. 51, 52) are abundant throughout the entire tissue, typical chloroplasts in the younger fruits, and chromoplasts in the older. In the mature fruit the contents of the plastids may

Granules and mitochondria are present in the cytoplasm in all flavedo and albedo cells (figs. 50, 51, 53).

OIL GLANDS.—The schizolysigenous development and the structure of the oil glands have been discussed in a number of earlier publications, as have, more recently, their distribution in relation to water spot (**2, 15, 35, 38, 41**).

VENATION.—In the rind, as in the fruit as a whole, the carpel is the unit of struc-

ture. The venation of the carpel is similar to that of the leaf, pinnate-reticulate, with conspicuous midrib and marginal veins (42). The pattern, however, is three-dimensional and may be traced in gross segments of the rind treated with phloroglucin and HCl. The ultimate veinlets bracket the oil glands and extend around them for half of their length (fig. 1). The component elements of the vascular tissue are similar to those of the phloem and xylem of the leaf. The xylem includes spiral vessels and tracheids, reticulate tracheids, and xylem parenchyma (34). As in the leaf the blind vein-endings consist of a knot of spiral tracheids only.

ALBEDO

On first examination of thin sections of albedo, no pattern of structure is distinguishable, but, when development of the tissue is followed in comparatively thick sections, the structural plan becomes clearly defined (figs. 1, 16–27).

Meristematic cells in the young albedo are typical tetrakaidekahedra in form. As they expand, they become spherical, and intercellular spaces appear (figs. 16, 19). Each single cell in this compact tissue is in contact and in direct plasmodesmatal connection with eight other cells of roughly equal size (fig. 18). As growth proceeds in the rind as a whole, the albedo cells fail to sustain the pace of cell division and expansion necessary for the formation of a solid tissue. The intercellular spaces thus expand enormously, and the cell outline becomes more and more deeply lobed, but throughout the entire period of growth the original plasmodesmatal connections are maintained (figs. 17, 18, 34).

With further growth in volume the lobes of the cell extend as arms approximately equal in length. Thus the open mesh of the mid-albedo in the mature fruit consists of typical eight-armed symmetrical cells. Each arm terminates in a sieve-plate or plasmodesmatal area. The symmetry of the cells is evident, since transverse, radial, and tangential sections of the mid-albedo are practically identical in appearance.

Variation in the rate of cell growth in different regions of the rind results in strains and stresses which cause deviation from the above typical pattern.

In the outer albedo, expansion is effected by continuous cell division rather than by increase in cell volume as in mid-albedo. The cells of this outer region differ from the typical eight-armed elements in the shortness of the tangential arms and in the absence of radial ones (figs. 28, 29).

In the inner albedo, cell divisions cease at approximately the same time as in mid-albedo. Arm development in the inner six to ten rows of cells is inhibited by radial compression (fig. 1).

The length of the cell arms in the rind as a whole varies with rind thickness and sponginess. The tissue pattern may be further complicated by the development of adventitious arms. The latter may arise at any point of the cell surface as papillae and may remain short (figs. 33, 34) or extend hypha-like into the intercellular spaces.

Walls throughout the entire albedo are comparatively uniform in thickness. The illusion of collenchyma-like thickenings adjacent to intercellular spaces observed in the necessarily thick sections of fresh material is an optical illusion due to perspective on the curving cell surfaces (figs. 32, 35).

The albedo cells are transparent, and abundant sugar may be demonstrated microchemically within the vacuoles. The peripheral cytoplasm, a thin layer,

extends throughout the arms and thence through the plasmodesmatal connections. The nucleus is generally central in position but occasionally may lie within one of the arms. Plastids are relatively few in number and in younger cells surround the nucleus, while in older cells they are more numerous in the peripheral cytoplasm. Mitochondria are evident in cells of all ages (fig. 53). The cells of the inner epidermis are of variable outline, thick-walled, and covered by a thin cuticle (fig. 1).

Cracking of rind

The cracking of the rind is the crucial stage of water-spot injury, since it allows increased penetration of water and also the entrance of mold spores (figs. 41–47). For intensive study of cracking, the tearing of the epidermis and of the underlying tissues may be induced more or less readily in maturing fruits by placing them in a rain chamber, or under running water, for a day or more. It may, however, be induced more consistently if segments of rind are placed, albedo downward, in water. The same effect is obtained by slicing off a sliver of rind from a mature fruit and standing the latter, wound downward, in a Petri dish of water. Within 1–3 hours cracks generally appear around the wound and radiate upward therefrom as the tissue becomes visibly waterlogged. The pattern of cracking induced in the peel by the above methods is similar to that seen in the field at the onset of water-spot injury.

The failure of the epidermis to keep pace with the volume increase of the underlying tissues is not peculiar to the mature fruit. In green oranges, ± 2 cm. in diameter, a relative lag in the rate of cell division and expansion may result in strain and incipient rupture of the middle lamella (figs. 41–43). At this stage of growth, however, deposition of cutin apparently is sufficiently active to prevent the development of an open wound. In fresh material stained with Sudan III, and in epidermal tissue isolated by maceration, such cutinized areas are readily observed. They may occur in any section of the epidermis, both near stomata and in the intervening areas. Occasionally, they lie immediately adjacent to one or more discolored, nonliving cells which, presumably, have been damaged by insect or other agency (fig. 42).

In mature fruits, also, similar incipient cracks, discernible only under high power or oil immersion, may be evident. As in younger fruits, cutin deposition may keep pace with underlying expansion, and further damage be prevented. If at last the epidermis is strained to the breaking-point, the initial split may occur at any point in the middle lamella, but thereafter, as the crack extends, it may rip through the center of living cells, leaving along the sides a fringe of moribund protoplasts and jagged remnants of wall.

The pattern of cracking appears on first sight entirely erratic, but, as expected, the basic plan depends on the topography of the skin—in particular, on the distribution of oil glands and stomata. As already observed, each oil gland is encircled by one or more orbits of stomata which lie along the slopes of the interglandular ridges. Conspicuous clefts appear first along the summits of the ridges, and, at the same time, furrows outline the gland margins (fig. 44). Finer cracks next arise around the accessory cells of the stomatal areas and also radiate in all directions to form a highly complex network. The depth of the cracks is variable and may be followed from the mere rupture of the in-

sufficiently elastic cuticle through the tearing of the epidermis and of the outer layers of the underlying flavedo (figs. 45–47).

If cracked fruits are left in comparatively dry air, wound healing follows in the normal way by cicatrization. Within 24 hours the entire network of a cracked surface is etched with a grayish-buff deposit (31), and a day or two later, in sections treated with Biebrich Scarlet or Sudan III, traces of cutin or suberin are apparent in the outer cell layers. Damaged cells adjacent to the cracks become shrunken and flattened, while ruptured oil glands, bereft of content, persist as minute empty cups. Suberization or cutinization is evident to a depth of one or more layers of cells. Beneath the cutinized surface, cell division is initiated in a phellogen, the cells of which are polygonal rather than cubical or rectangular as in the typical cork of the stem.

Lignification of epidermis

Sporadically, in mature fruits of all sizes, generally toward the end of the growing season, lignification may occur in small areas of the epidermis. The initial stimulus for this fundamental chemical change in cell-wall composition has not been determined. The existence of such comparatively nonextensible areas probably affects the tendency to crack in some fruits. As a symptom, however, it does not appear to be widespread.

Absorption of aqueous stains

The ready absorption of water by *Citrus* rind is doubtless a potent factor in water-spot injury (23). Absorption may be readily demonstrated by immersion of mature fruits for varying periods of time (2 hours to overnight) at different temperatures (±65° F., ±28° F.) in 0.01% aqueous methylene blue. Qualitatively, the results produced by brief and by prolonged staining are essentially similar, though accumulation of dye is naturally intensified after the longer immersion.

That the permeability of epidermal cells is variable is evident from the following preliminary observations:

1. The staining of cell vacuoles is well marked in outer accessory cells around older stomata, whereas in inner younger accessories it may be faint or absent. All accessory cells, as already noted, are thinly cutinized. Stomatal plugs, when present, are invariably dark blue in color, while the guard cells may or may not show dye absorption (figs. 54, 55).

2. The permeability of younger cells is, in general, erratic. Accessories and guard cells around younger stomata may or may not be faintly or heavily stained. In the interstomatal areas dye penetration is sporadic and evident chiefly in older cells. The age of cells is indicated by the uniformity of thickness of the radial walls, in contrast to the disparity obvious in cells which have recently divided.

3. Oil-gland cover cells of older and younger glands are consistently stained. In radial section dye penetration is evident in both gland-sheath and glandular cells.

4. Wherever visible cracks occur, the staining of epidermal and of underlying cells is immediate and intense. Minute cracks in the middle lamella, detected only microscopically, appear as discrete, deeply stained, pinpoint areas (fig. 56).

Some effects of freezing

During winter, damage by occasional frosts may not be effectively prevented by smudging. The specific susceptibility of the fruit rind cells to low temperature has been discussed in detail in a recent

paper (28). The effect of low temperature in the field in relation to water-spot injury is still a matter for physiological investigation. As far as the structure of the rind as a whole is concerned, the result of intense or prolonged freezing is tissue death (5, 27). Oil glands are ruptured, cracks appear, and water loss causes cell shrinkage. Beneath the depressed, discolored, shrunken cutinized or suberized areas of frost damage, a phellogen is initiated, the activity of which, however, appears to be limited to one or two tangential divisions only. Frost injury similar to that in the field may be induced experimentally in picked fruit by freezing localized areas of the rind.

In addition to visible surface damage, freezing results in internal tearing. Rupture occurs generally between hypodermis and flavedo and is evident both in field and in experimentally frozen fruit.

A curious phenomenon which can best be described as "sweating" occurs when fruits are first removed from a refrigerator (±28° F.) to warm air (±70° F.). Freshly picked fruits are placed in a refrigerator and dotted with drops of water which are marked by rings of India ink. In a short time the water drops freeze, but within 24 hours the ice evaporates. On removal from the refrigerator, the entire surface of the orange, when immediately and carefully examined with a hand lens, appears at first uniformly dull. Within 60 seconds, however, the water-drop areas glisten with moisture. The liquid exudes from apparently intact epidermal or accessory cells, not from oil glands, nor through stomata, nor through microscopically observable surface cracks. The fluid is sufficiently profuse to ooze downward and collect temporarily in the depressions of the oil glands. In dry air the moisture evaporates in a short time and leaves an intact epidermal surface. This "sweating" is not to be confused with the normal condensation of moisture which occurs over the chilled fruit within the next 5 minutes after removal from the cold temperature.

The explanation of sweating is still to be sought, perhaps in the intricate structure of the cell wall. The walls of living cells are saturated with water. On freezing, differential expansion in the intermicellar spaces may result in micel dislocation and the formation of submicroscopic clefts. Through such channels in the weakened wall, on thawing which follows immediately after release to warm air, surplus fluid "sweat" escapes. The nature of the exudate is undetermined but is presumably water with a trace of natural oil.

Discussion

In regard to the orchard practice of oil-spraying, it is established that the incidence of water spot is consistently higher in sprayed than in fumigated or untreated groves (10, 11, 12, 14, 37). Oil is known to penetrate the orange tissues (1, 17, 25, 32), but no anatomical differences (e.g., hypertrophy or dwarfing of cells, meristematic activity, etc.) were observed in the unsprayed, sprayed, or even oil-dipped fruits. The presence of oil in the walls and in the protoplasts of epidermal and underlying cells may affect their physiological condition, but no significant differences in permeability were indicated in preliminary experiments on dye absorption.

Further investigation may show that specific cells of the rind, after oil-spraying, are less tolerant to low temperature than untreated cells (28). The presence of frost-killed cells would prove an additional factor in rind weakness, since it

would increase permeability and tendency to crack.

Apart from possible oil-spray damage, the rind of the Washington navel is structurally weak, owing to scattered areas of lightly cutinized cells in the epidermis. Chemical analyses of the fruit as a whole indicate that, during ripening, sugar and nitrogen content approach a maximum (3, 4, 19, 36) and that, at the same time, significant changes occur in the pectic substances of the cell walls (16, 30, 33). Thus both osmotic conditions and imbibition capacity of the wall favor water absorption and retention. At maturity, therefore, the balance between epidermal extension and volume increase is critical but, under normal weather conditions, is maintained. If, however, heavy rains, alternating or not with frosts, occur during this period, the physiological equilibrium may be upset, and excessive water intake and localized accumulation stretch the epidermis to the cracking-point.

Recent work indicates that all varieties of navel oranges are not equally susceptible to water spot (21). Some, less desirable from the commercial standpoint, are more resistant than the Washington navel. No significant anatomical differences were observed in the rind structure of susceptible and nonsusceptible varieties.

Summary

1. The symptoms of water-spot injury, as already described by other investigators, are waterlogging and subsequent cracking of the rind of the ripening Washington navel orange.

2. The epidermis of the rind of the Washington navel, as of other varieties, is structurally weak. Cell division continues during maturity, and discrete, new, thin-walled cells are consistently present. In addition, the thinly cutinized accessory cells constitute zones of weakness.

3. That the epidermis and underlying tissues are readily permeable to water is demonstrated by immersion in vital stains. Methylene blue appears in the cell vacuoles, particularly of accessory cells and gland-cover and gland-sheath cells.

4. The sponginess of the rind, especially of the albedo, facilitates water accumulation.

5. The albedo differentiates from tetrakaidekahedral meristematic cells and, when mature, consists of an open meshwork of eight-armed cells interconnected by plasmodesmata.

6. Structural weakness is already apparent in the young fruit. Incipient microscopic cracks, owing to normal growth strains or insect puncture, are, however, healed by concurrent deposition of cutin.

7. In the mature fruit the balance between epidermal extension and increase in volume of underlying tissues is critical and may be upset by excessive water absorption during winter rains. The cracks which result may heal in dry weather by normal cicatrization or, in continued rain, may remain open and allow the entrance of fungal spores.

8. Why the Washington navel orange is more susceptible to water spot than other varieties which ripen at the same time is undetermined. No significant differences in rind structure are evident.

9. The orchard practice of oil-spraying does not visibly affect rind structure. No anatomical difference is observed between untreated and oil-sprayed, or even oil-dipped, fruits.

DIVISION OF BOTANY
UNIVERSITY OF CALIFORNIA
LOS ANGELES

LITERATURE CITED

1. BARTHOLOMEW, E. T. The effects of oil sprays on plant tissues. Proc. Third Western Shade Tree Conf. 3:53–61. 1936.
2. BIERMANN, M. Beiträge zur Kenntniss der Entwickelungsgeschichte der Früchte von *Citrus vulgaris* Risso und anderer *Citrus* arten. Inaugural Dissertation Univ. Bern. 1896.
3. CAMERON, S. H.; APPLEMAN, DAVID; and BIALOGLOWSKI, J. Seasonal changes in the nitrogen content of citrus fruits. Proc. Amer. Soc. Hort. Sci. 33:87–89. 1935.
4. CHACE, E. M., and CHURCH, C. G. Maturity data on the California Washington navel orange. Calif. Citrograph 15:534, 569. 1930.
5. CHANDLER, W. H., and HILDRETH, A. C. Evidence as to how freezing kills plant tissue. Proc. Amer. Soc. Hort. Sci. 33:27–35. 1936.
6. CITRUS EXPERIMENT STATION, RIVERSIDE. Unpublished data.
7. CRAFTS, A. S. A technique for demonstrating plasmodesma. Stain Technol. 6:127–129. 1931.
8. DE BARY, A. Über die Wachsüberzüge der Epidermis. Bot. Ztg. 29:128–139. 1871. (Total pages 128–619 et. seq.).
9. DOUS, E. Über Wachsausscheidungen bei Pflanzen. Bot. Arch. 19:461–473. 1927.
10. EBELING, W. Effect of spray material on citrus trees. Calif. Citrograph 21:370, 409. 1936.
11. EBELING, W., and KLOTZ, L. J. The relation of pest control treatment to water rot of navel oranges. Calif. Dept. Agr. Mo. Bull. 25:360–368. 1936.
12. EBELING, W.; KLOTZ, L. J.; and PARKER, E. P. Experiments on navel water rot. Calif. Citrograph 23:410, 434–436. 1938.
13. FAWCETT, H. S. The recent decay of navel oranges. Calif. Citrograph 12:219. 1927.
14. FAWCETT, H. S.; KLOTZ, L. J.; and HAAS, A. R. C. Water spot and water rot of citrus fruits. Calif. Citrograph 18:165, 175. 1933.
15. FORD, E. S. Anatomy and histology of the Eureka lemon. BOT. GAZ. 104:288–305. 1942.
16. GADDUM, L. W. The pectic constituents of citrus fruits. Fla. Agr. Exp. Sta. Bull. 268:1–23. 1934.
17. GINSBERG, J. W. Penetration of petroleum oils into plant tissues. Jour. Agr. Res. 43:469–474. 1931.
18. GUILLIERMOND, A., and GAUTHERET, R. Recherches sur la coloration vitale des cellules végétales. Librairie générale de l'enseignement, Paris. 1940.
19. HARVEY, E. M., and RYGG, G. L. Physiological changes in the rind of California oranges during growth and storage. Jour. Agr. Res. 52:723–746. 1936.
20. KLOTZ, L. J. Water damage to a citrus relative, *Fortunella margarita*. Phytopath. 29:214–215. 1939.
21. ———. Unpublished data.
22. KLOTZ, L. J., and BASINGER, A. J. The influence of various types of rind injury on the incidence of water spot of navel oranges. Calif. Dept. Agr. Mo. Bull. 27:232–241. 1938.
23. KLOTZ, L. J., and HAAS, A. R. C. Some differences between button-blossom halves of citrus fruits. Calif. Citrograph 18:318, 324. 1933.
24. KLOTZ, L. J., and TURRELL, F. M. Rind structure and composition in water spot of navel orange. Calif. Citrograph 25:45, 56, 57. 1939.
25. KNIGHT, H. A microtechnique for observing oil penetration in citrus leaves after spraying. Science 68:572. 1928.
26. LA RUE, C. Intumescences on poplar leaves. Amer. Jour. Bot. 20:1–17, 159–175. 1933. *Ibid.* 23:520–524. 1936.
27. LEVITT, J. Frost killing and hardiness of plants. Burgess Publ. Co., Minneapolis. 1945.
28. LEVITT, J., and NELSON, R. C. Relative resistance of morphologically different orange peel cells to various injury factors. Biodynamica 4:57–64. 1942.
29. LINSBAUR, K. Handbuch der Pflanzenanatomie 4:111–124. 1930.
30. POORE, H. D. Citrus pectin. U.S. Dept. Agr. Bull. 1323. Washington, D.C. 1925.
31. PRIESTLEY, J. H., and WOFFENDEN, L. M. Causal factors in cork formation. New Phytologist 21:252–268. 1922.
32. ROHRBAUGH, P. W. Penetration and accumulation of petroleum spray oils in the leaves, twigs, and fruit of citrus trees. Plant Physiol. 9:699–730. 1934.
33. RYGG, G. L., and HARVEY, E. M. Behavior of pectic substances and naringin in field and in storage. Plant Physiol. 13:571–586. 1938.
34. SCOTT, F. M., and SCHROEDER, MARY. Unpublished data.
35. SIECK, W. Die schizolysigenen Sekretbehälter. Jahrb. Wiss. Bot. 27: 197–242. 1895.
36. SINCLAIR, W. B.; BARTHOLOMEW, E. T.; and EBELING, W. Comparative effects of oil spray and hydrocyanic acid fumigation on the composition of orange fruits. Jour. Econ. Ent. 34: 821–829. 1941.
37. SMITH, R. H. Observations on the reactions of citrus trees to oil sprays. Citrus Leaves 12:6–8. 1932.
38. SOLEREDER, H. Systematic anatomy of dicotyledons. *Trans.* L. A. BOODLE and F. E. FRITSCH. Clarendon Press, Oxford. 1908.
39. TSCHIRCH, A., and OESTERLE, O. Anatomische Atlas der Pharmocognosie und Nährungsmittelkunde. Leipzig. 1898.
40. TUNMANN, O., and ROSENTHALER, L. Pflanzenmikrochemie. Gebrüder Borntraeger, Berlin. 1931.
41. TURRELL, F. M., and KLOTZ, L. J. Density of stomata and oil glands and incidence of water spot in the rind of Washington navel orange. BOT. GAZ. 101:862–871. 1940.
42. WEBBER, H. J., and BATCHELOR, L. D. The citrus industry, Vol. I: History, botany and breeding. Pp. 680–705. Univ. Calif. Press, Berkeley and Los Angeles. 1943.

CITRUS LEAF STOMATA: STRUCTURE, COMPOSITION, AND PORE SIZE IN RELATION TO PENETRATION OF LIQUIDS[1]

F. M. TURRELL

Introduction

Stomatal numbers and sizes have received considerable attention in plant investigations. Either number or size, or both, have been used in theoretical considerations of transpiration (8, 17), diffusion rates (3), genetic composition (7), age of clones (2), height of leaf insertion on the stem (15, 25), xeromorphy (15), differentiation of sun- and shade-leaf development (4), ecological habitat (18), irrigation (16), and inorganic nutrition (13). In some cases stomatal pore size has been reported, but in much of the work stomatal size is reported as guard-cell size.

REED and HIRANO (19) and HIRANO (9) have made excellent and extensive stomatal counts in citrus leaves; but no estimates have been made of stomatal pore sizes in citrus leaves, though pore measurements were made by ECKERSON (6) for a rather sizable number of other genera.

Stomatal pore size in citrus leaves is of practical importance because penetration of sprays used for control of insect and fungus pests of citrus may be a function of pore size, shape, and conformation, as well as of spray composition. This conclusion is fortified by consideration of the well-known formula for the rise of a liquid in a capillary tube

$$h = \frac{2\gamma \cos \theta}{r g}, \qquad (1)$$

where h is the height of rise (i.e., a measure of the attractive force involved), γ is the surface tension of the liquid, θ is the contact angle, r is the radius of the tube, and g is gravitational force. These relationships were discussed by HOSKINS (11) and by WILLOWS and HATSCHEK (24).

Material and methods

Stomatal structure was studied from samples cut from fresh citrus leaves, which were killed in formalin–acetic acid–alcohol and made into permanent microscope slides by standard methods. Both transverse and tangential sections were examined with a microscope fitted with an oil-immersion objective. Photographs and drawings were made and used as a basis in making a plastic model of a stoma for interpreting the external stomatal structure.

For the studies on stomatal pore size, five average-sized sun leaves were selected from one tree each of Marsh grapefruit, Valencia orange, and Washington navel orange; ten sun leaves, five medium-sized and five small-sized, were selected from a Eureka lemon tree. All the trees were about 12 years old, in good condition, and growing in plots at the University of California Citrus Experiment Station, Riverside. The leaves were picked fresh as needed and were washed with water. The resinous stomatal plugs were removed with alternate applications of alcohol and xylene, using a cotton swab. After killing in boiling alcohol, freehand tangential sections of the lower epidermis were made, stained in ruthenium red, mounted in glycerine, and subjected to a direct microscopical examination, using a high-power (62×) dry ob-

[1] Paper no. 557, University of California Citrus Experiment Station, Riverside, Calif.

jective, in combination with a 12× ocular fitted with an ocular micrometer. The dimensions of ten or more stomatal pores were measured on each leaf. Calibrations indicated that one division of the ocular micrometer was equal to 1.18 μ. Readings could be made to one-half of a division, giving an accuracy of about 0.59 μ.

The ellipticity of the stomatal pores was determined by making enlargements (4400×) of camera lucida drawings of stomatal pores and by testing points (p) on the curve in the first quadrant by direct measurement of r and r_1 and comparison with calculated values, using the observed major (a) and minor (b) semiaxes of the pore to determine the foci (c) for measured and calculated values (fig. 1). The points (p) were determined by erecting perpendiculars to the x-axis at arbitrarily set points, $x_1 \ldots . x_n$. The value c was calculated from the formula

$$c = \sqrt{a^2 - b^2},$$

while r and r_1 were calculated, respectively, from the formulas

$$r = a + \frac{cx}{a} \quad \text{and} \quad r_1 = a - \frac{cx}{a}.$$

Stomatal pore perimeter and area were calculated from the formulas for the perimeter (P) and area (A) of an ellipse

$$P = 2\pi\sqrt{\frac{a^2 + b^2}{2}} \quad \text{and} \quad A = \pi a b,$$

where a is the major semiaxis (length) and b the minor semiaxis (width).

Results

It was determined by the methods of mathematical analysis described above that stomatal pores of Washington navel orange leaves are elliptical. The observed elliptic constants, and the calculated and observed variables, are shown in table 1.

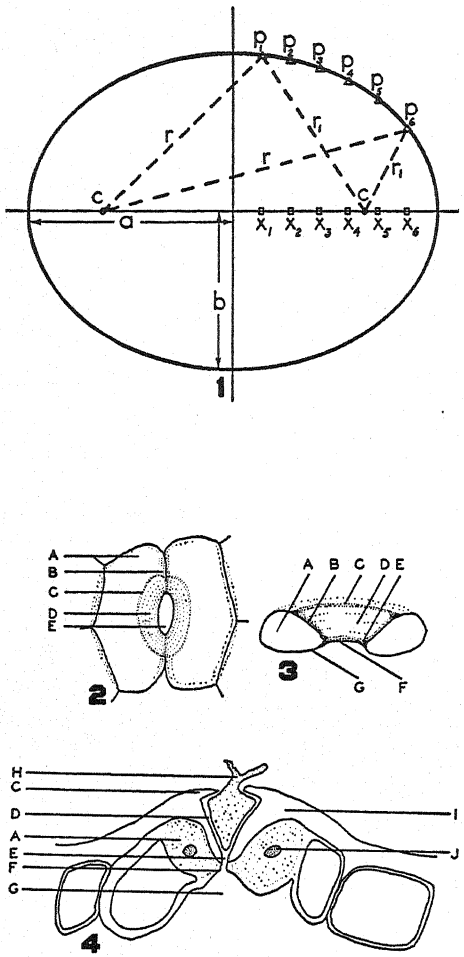
TABLE 1

COMPARISON OF CALCULATED AND OBSERVED VALUES OF r AND r_1 FOR VARIOUS VALUES OF x, FOR STOMATAL PORE I, WHEN a = 2.05, b = 0.7 (OBSERVED), AND c = 1.93 (CALCULATED); AND FOR STOMATAL PORE II, WHEN a = 2.28 AND b = 0.62 (OBSERVED), AND c = 2.19 (CALCULATED)*

p SUBSCRIPT	x	PORE I				PORE II			
		r		r_1		r		r_1	
		Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
1	0.50	2.52	2.50	1.58	1.52	2.76	2.75	1.80	1.80
2	1.00	2.99	2.99	1.11	1.10	3.24	3.24	1.32	1.32
3	1.50	3.46	3.46	0.64	0.65	3.72	3.74	0.84	0.86
4	1.93	3.86	3.82	0.24	0.30	.....	.....	.....	.....
5	2.00	.....	.....	.....	.....	4.20	4.19	0.36	0.38

* For explanation of symbols and methods see text.

The general structure of a stoma of a Valencia orange leaf is shown in figures 2–4. Notable features are the ridges surrounding the stomatal chamber, the loosely fitting stomatal plug which nearly fills the stomatal chamber (the space between the plug and the chamber wall is about 0.5 μ), and the extremely large stomatal chamber (7.8 μ deep), at the bottom of which lies the elliptical stomatal pore. BROWN and ESCOMBE (3) found the stomatal pore ("tube") in *Helianthus* to be 14 μ in length. In the



FIGS. 1–4.—Fig. 1, diagram of an ellipse, showing method used for testing ellipticity of stomatal pore: a, major semiaxis; b, minor semiaxis; c, foci; x_1, x_2 . . . . , arbitrary values on x-axis; p_1, p_2 . . . . , points cut off on ellipse by perpendiculars erected at x_1, x_2 . . . . ; r, distance from $-c$ to p; r_1, distance from $+c$ to p. Fig. 2, diagram of surface view of stoma of Valencia orange leaf: A, guard cell; B, stomatal crevice; C, stomatal ridge; D, stomatal chamber (outer vestibular opening—*Vorhoff*); and E, stomatal pore. Fig. 3, diagram of longitudinal cross section of stoma of Valencia orange leaf: F, stomatal pore wall; G, substomatal chamber (inner vestibular opening—*Hinterhoff*). Fig. 4, diagram of transverse section through stoma of Valencia orange leaf, at center: H, stomatal plug; I, cuticle; and J, nucleus.

Valencia orange leaf, however, the walls of the stomatal pore are only 3.1 μ in length, so that even though the substomatal chamber formed by the inner side of the guard cells is shallow, the stomatal pore appears to be a perforation in a thin membrane formed by an extension or protrusion of the inner walls of the guard cells. On the outer surface the line of junction of the guard cells is somewhat sunken and forms a miniature crevice leading to the stomatal chamber. This crevice is not interrupted by the stomatal ridge lying along the edge of the stomatal chamber, so that a line of flow apparently exists from the surface to the interior of the stoma. A cuticle about 3 μ thick covers the outer surface of the guard cells, forms the stomatal ridge, and extends downward into the stomatal chamber to the entrance of the stomatal pore. Although the stomatal chamber is completely cutinized, the stomatal pore walls are not. The pore walls react strongly with ruthenium red, and this suggests that they may be constituted either entirely or in part of pectinaceous material. Examination of the pore region with a polarizing microscope showed only slight and occasional streaks of double-refractive material. This seems to substantiate the staining evidence that the walls of the stomatal pore are pectinaceous. The stomatal plug, according to other investigations (10), seems to be of resinous composition.

The average lengths ($2a$) and widths ($2b$), with standard errors, of fifty or more stomatal pores of leaves of each of the common commercial varieties of citrus are shown in table 2, in conjunction with the maximum and minimum pore lengths and widths. In the leaves of Eureka lemon the difference between pore lengths of medium and small leaves is perhaps not significant, but pore

width appears significantly smaller in the small leaves. Average stomatal pore length was smallest in the leaves of Washington navel orange, and larger in increasing order in Eureka lemon, Marsh grapefruit, and Valencia orange. Pores of small leaves of Eureka lemon and of medium-sized leaves of Marsh grapefruit, though about equal in width, were narrower than those of Washington navel orange. Leaves of Washington navel orange had the second smallest average pore width. Pores of medium-sized leaves of Eureka lemon were next larger in width, and those of Valencia orange were largest. Stomatal pore size was highly variable, Marsh grapefruit and Washington navel orange showing the least variability, Eureka lemon and Valencia orange the most.

TABLE 2

STOMATAL PORE SIZES OF CITRUS LEAVES

Kind of leaf	Number of pores measured	Leaf size	Pore length (μ)			Pore width (μ)			Average pore perimeter* (μ)	Average pore area* (μ²)
			Average	Maximum	Minimum	Average	Maximum	Minimum		
Eureka lemon...	50	Medium	7.04±1.73†	12.4	3.5	3.08±0.97†	5.9	1.2	17.1	17.0
Eureka lemon...	50	Small	7.13	12.4	2.4	2.07	5.9	1.2	16.5	11.6
Marsh grapefruit	50	Medium	8.06±1.59	12.0	4.7	2.08±0.50	3.5	1.2	18.5	13.2
Valencia orange.	70	Medium	8.91±3.10	14.2	5.3	3.80±0.98	6.5	1.8	21.5	26.6
Washington navel orange....	50	Medium	4.78±0.86	8.3	3.5	2.32±0.82	4.7	1.2	11.8	8.7

* Considering the stomatal pore as an ellipse.

† Standard error.

In order, from smallest to largest, were those of Washington navel orange, of small leaves of Eureka lemon, medium-sized leaves of Eureka lemon, Marsh grapefruit, and Valencia orange (table 2). For pore areas the order of size was the same, except that pore areas of Marsh grapefruit were smaller than those of medium leaves of Eureka lemon.

Discussion

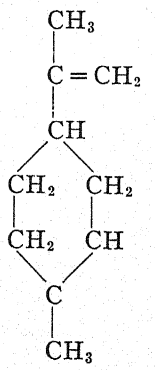
The term "stoma" is derived directly from the Greek στομα, meaning "mouth." JACKSON (12) defines it as "a breathing pore or aperture in the epidermis, surrounded by two guard-cells, leading into an intercellular space communicating with internal tissue." From this definition it may be inferred that "stoma" applies to the pore between two adjacent guard cells. Generally, however, as indicated in papers already cited, the guard cells have been included as part of the stoma. The nomenclature used in the present paper has, so far as possible, been based on common usage in the literature. Thus "stoma" has been employed in the general sense of including a stomatal pore and two guard cells. In a special sense, the stomatal pore has been limited in this paper to mean the connecting space between the stomatal chamber and the substomatal chamber. The terms "stomatal ridge," "stomatal crevice," and "stomatal plug" have been employed without precedent in order to facilitate discussion of stomatal structure in relation to spray penetration.

The structure of the citrus stoma is such that *a liquid of the proper charac-*

teristics might, if placed on the surface of the leaf, be drawn rapidly into the mesophyll. Liquid accumulating in the narrow stomatal crevice, through action of the various vectors of gravitational force operating when the leaf is moved by air currents, could be drawn to the edge of the stomatal plug, where it would be drawn rapidly under by capillary force, even though the stomata are confined to the lower leaf surface (except over the major veins) where entrance is hindered by the action of gravitational force. Inasmuch as the distance between the plug and the stomatal chamber wall is about half a micron, the capillary force might be sizable. Factors that would be expected to influence capillary flow, other than pore size, are the pectic nature of the constituents of the stomatal tube wall (21), the elliptical pore of small width (averaging 2–4 μ), the resinous stomatal plug, and the cuticular covering of the stomatal chamber. Rate of flow for a particular liquid would, of course, be influenced by the magnitude of the molecular forces involved in the liquid-solid interfaces, as well as by the size of the free space in the channel between the stomatal plug and chamber wall, and by the pore area.

In the monsoon region of forests of China and the Malay Archipelago (22), the problem of gas diffusion into and out of the citrus leaf mesophyll could be critical during periods of intense insolation immediately following heavy rains, if the stomata and intercellular spaces of the mesophyll became filled with water. The likelihood of the latter occurrence is probably small, however, as the structure of the citrus stoma seems well adapted to the native habitat of the cultivated species of *Citrus*. Resinous stomatal plugs and cutinized stomatal chambers perhaps effectively prevent the stomata, and consequently the intercellular spaces, from filling with water.

The operation of the mechanism in preventing water penetration may be explained as follows: The contact angle (θ) between distilled water and waxed capillary glass tubes, as has been shown by EBELING (5), is large (i.e., cos θ is small), and the height of rise of water in the tubes is zero; whereas in clean unwaxed capillary glass tubes the contact angle θ is small, and the height of rise is more than 2 cm. This shows that, where contact angles are large, surface forces are small. Inasmuch as the citrus resin (of which the citrus stomatal plug consists) is probably formed by polymerization and oxidation (of low order) of the ethereal oils, as are most resins, one might expect a citrus resin to be polymerized from a common ethereal oil such as limonene, which is found in citrus. The structural formula indicates that this substance is relatively nonpolar and that the contact angle (θ) of water with it would be large.



Surface forces are known to be the result, in part at least, of the structures of the molecules making up the surface. The 1,8(9)-p-menthadiene (limonene), though not so symmetrical as the parent molecule menthane, electrically, is fairly symmetrical. There are no O-H, N-H, S-H, or halogen bond moments to con-

tribute large charges. Such charges as would effect polarity arise primarily around the *1* and *4* carbons of the ring and from C-H bonds. Rough calculations of the bond moments by differences of electro-negativities (23) indicate a moment of about 1.6 Debye units, which is relatively small and indicates that the water contact angle with limonene and its polymers would be large.

MARKLEY *et al.* (14) have isolated and identified some of the constituents of grapefruit peel cuticle and found solid fatty acids having a mean molecular weight corresponding to $C_{32}H_{64}O_2$; the unsaturated fatty acids linolenic ($C_{17}H_{29}COOH$), linoleic ($C_{17}H_{31}COOH$), and oleic ($C_{17}H_{33}COOH$); a sapogenic ketone, $C_{30}H_{52}CO$; hydrocarbons $C_{29}H_{60}$ and $C_{31}H_{64}$; a phytosterol, $C_{28}H_{47}OH$; and umbelliferone, $C_9H_6O_3$. These compounds, likewise, obviously have a very small degree of polarity (as the number of OH and CO groups are few), and the contact angle (θ) of water with them therefore must be large. EBELING (5) has investigated the contact angles of water on the intact ventral surfaces of various citrus leaves and found them to be high (orange, 69°54′; grapefruit, 67°21′; lemon, 76°42′). The fact that there is such a small space (0.5 μ or less) between the stomatal plug and the stomatal chamber wall suggests that strong capillary forces might be active (as may be seen by making r very small in eq. [1]). However, when the contact angle is large, cos θ is small; this indicates that surface forces will be small (as may be seen by making cos θ small in eq. [1]) and that any significant penetration of citrus stomata by water is unlikely. In fact, TURRELL and KLOTZ (21) found that, although large and small oranges have statistically significant differences in the number of stomata in the peel, there were no significant differences in the amount of water absorbed when the fruits were immersed.

It seems, therefore, that the above explanation serves to show why the water-spotting of Washington navel orange fruit is a result of water penetration of the peel through cracks and abrasions and not through stomata. With oil sprays, the contact angles (5) may be moderately low ($\theta = 32°5'$) to extremely low ($\theta = 11°26'$), and penetration of the stomata may be expected. With water sprays containing certain spreading and wetting agents, contact angles in tests on *Viburnum* leaves (5) were moderately low (45°22′) to fairly low (27°57′). There is some evidence that, so far as oil sprays are concerned, penetration of citrus stomata has occurred. ROHRBAUGH (20) observed that, when oil was painted on the under surfaces of orange leaves, it penetrated into the mesophyll more rapidly than when painted on the upper surfaces, owing to entrance through the stomata, which are present only on the underside of the blade.

Penetration of the leaf by zinc, iron, manganese, and other salts carried in emulsions in oil sprays, or in water sprays containing certain wetting or spreading agents, may be expected to enter the stomata rather readily. Certain spreading or wetting agents may be more effective than others, however. EBELING (5) has shown that the contact angle (θ) varies with the wetting agent and with the kind of waxy substrate.

In view of the peculiar structure of the plugged citrus stoma, questions naturally arise: Are the stomata operative? If they are, how much are pore dimensions affected? Transpiration measurements by BARTHOLOMEW (1) showed a light-to-shade decrease in dorsal/ventral transpiration ratio from 86 mg./752 mg. to

52 mg./227 mg. of water lost. On the reversed sequence (shade to light), the ratios were 43 mg./252 mg. to 69 mg./684 mg. This strongly suggests that the stomata are operative, since the effects of temperature were clearly largely eliminated by the change in sequence.

In view of the heavy cuticular layer over the guard cells in the region of the stomatal chamber (fig. 4), it seems unlikely that the chamber dimensions would vary greatly. The stomatal plug so nearly fills the stomatal chamber that closure of more than 1 or 2 μ of this portion of the stomata would be impossible. However, this closure, though involving only a small movement, might for all practical purposes stop gaseous exchange.

Examination of figure 4 indicates that the closure of the stomatal pore might easily be effected by the swelling of the guard cells, for the guard-cell walls are quite thin in the vicinity of the pore and quite thick elsewhere. We therefore may expect some variation in pore size with time of day. The leaves used in the present study were picked generally between 9:00 and 10:00 A.M. and not later than 2:00 P.M. Correlation of stomatal pore size in citrus with time of day remains to be studied.

Analysis of variance for the significance of the differences among stomatal pore lengths and pore widths for the various kinds of citrus leaves is shown in table 3. Computation of the F values for pore lengths from this table gave 46.7. Computation of the F values for pore widths for the various kinds of citrus gave 275.15. Thus, both pore lengths and pore widths are significantly different at the 1% level for the different kinds of citrus leaves.

TABLE 3

ANALYSIS OF VARIANCE OF STOMATAL PORE LENGTH AND WIDTH IN VARIOUS KINDS OF CITRUS LEAVES

Source of variation	Degrees of freedom	Sum of squares		Mean square	
		Pore length	Pore width	Pore length	Pore width
Total	219	971.7	1803	4.44	8.23
Between means of kinds	3	381.0	1428	127.00	476.00
Within means of kinds	217	590.7	375	2.72	1.73

Summary

1. The stomatal pore in Washington navel orange leaves is elliptical in shape. The average stomatal pore lengths and widths for medium-sized leaves of Eureka lemon were 7.04 × 3.08 μ; of Marsh grapefruit, 8.06 × 2.08 μ; of Valencia orange, 8.91 × 3.80 μ; and of Washington navel orange, 4.78 × 2.32 μ. Stomatal pore length and width were both highly significantly different for each kind of leaf.

2. Study of a stoma of a lemon leaf shows that it has a complicated surface structure which makes calculation of the action of surface forces on spray liquids more difficult than if based on stomatal pore size alone. Evidence based on molecular forces indicates that citrus stomata are not penetrated by water but may be penetrated by oil.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BARTHOLOMEW, E. T. Certain phases of citrus leaf transpiration. Amer. Jour. Bot. **18**:765–783. 1931.
2. BENEDICT, H. M. Senile changes in leaves of *Vitis vulpina* L. and certain other plants. N.Y. (Cornell) Agr. Exp. Sta. Mem. **7**:273–370. 1915.
3. BROWN, H. T., and ESCOMBE, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Roy. Soc. (London), Ser. B, **193**:223–291. 1900.
4. DOI, TOHEI. Über die Sonnen- und Schattenblätter einiger Bäume. Tokyo Imp. Univ. Col. Sci. Jour. **40**:1–36. 1917.
5. EBELING, WALTER. The role of surface tension and contact angle in the performance of spray liquids. Hilgardia **12**:665–698. 1939.
6. ECKERSON, S. H. The number and size of the stomata. BOT. GAZ. **46**:221–224. 1908.
7. FROST, H. B., and KRUG, C. A. Diploid-tetraploid periclinal chimeras as bud variants in citrus. Genetics **27**:619–634. 1942.
8. GIDDINGS, L. A. Transpiration of *Silphium laciniatum*. Plant World **17**:309–328. 1914.
9. HIRANO, E. Relative abundance of stomata in citrus and some related genera. BOT. GAZ. **92**: 296–310. 1931.
10. HODGSON, R. W. Some abnormal water relations in citrus trees of the arid southwest and their possible significance. Calif. Univ. Pub. Agr. Sci. **3**:37–54. 1917.
11. HOSKINS, W. M. The penetration of insecticidal oils into porous solids. Hilgardia **8**:49–82. 1933.
12. JACKSON, B. D. A glossary of botanic terms, with their derivation and accent. 4th ed. J. B. Lippincott Co., Philadelphia. 1928.
13. LUTMAN, B. F. Cell size and structure in plants as affected by various inorganic elements. Vermont Agr. Exp. Sta. Bull. **383**:1–54. 1934.
14. MARKLEY, K. S.; NELSON, E. K.; and SHERMAN, M. S. Some wax-like constituents from expressed oil from the peel of Florida grapefruit, *Citrus grandis*. Jour. Biol. Chem. **118**:433–441. 1937.
15. MAXIMOV, N. A. The plant in relation to water. Edited with notes, by R. H. YAPP. George Allen & Unwin, Ltd., London. 1929.
16. MAXIMOV, N. A., and ZERNOVA, L. K. Behavior of stomata of irrigated wheat plants. Plant Physiol. **11**:651–654. 1936.
17. MUENSCHER, W. L. C. A study of the relation of transpiration to the size and number of stomata. Amer. Jour. Bot. **2**:487–504. 1915.
18. PENFOUND, W. T. Plant anatomy as conditioned by light intensity and soil moisture. Amer. Jour. Bot. **18**:558–572. 1931.
19. REED, H. S., and HIRANO, E. The density of stomata in citrus leaves. Jour. Agr. Res. **43**:209–222. 1931.
20. ROHRBAUGH, P. W. Penetration and accumulation of petroleum spray oils in the leaves, twigs, and fruit of citrus trees. Plant Physiol. **9**:699–730. 1934.
21. TURRELL, F. M., and KLOTZ, L. J. Density of stomata and oil glands and incidence of water spot in the rind of Washington navel orange. BOT. GAZ. **101**:862–871. 1940.
22. WEBBER, H. J., and BATCHELOR, L. D. (eds.). The citrus industry. Vol. I, History, botany, and breeding. Pp. 51, 129, 135, 211, 341, 387. University of California Press, Berkeley, Calif. 1943.
23. WHELAND, G. W. The theory of resonance and its application to organic chemistry. John Wiley & Sons, Inc., New York. 1944.
24. WILLOWS, R. S., and HATSCHEK, E. Surface tension and surface energy and their influence on chemical phenomena. 2d ed. J. & A. Churchill Co., London. 1919.
25. YOCUM, L. E. The stomata and transpiration of oaks. Plant Physiol. **10**:795–801. 1935.

PIGMENT GLANDS OF COTTONSEED. II. NATURE AND PROPERTIES OF GLAND WALLS

CHARLOTTE H. BOATNER, CATHERINE M. HALL, MARY L. ROLLINS, AND LEAH E. CASTILLON

Introduction

Gossypol, the principal pigment of cottonseed, is a hexaphenolic, dicarbonyldinaphthalene derivative (1, 8, 22) which cannot be assigned to any of the more important classes of plant pigments such as porphyrins, carotenoids, flavones, anthocyanins, or quinones (15, 18). It has been stated to be peculiar to seed of the genus *Gossypium* and a few other closely related genera (26, 31). The recently discovered purple- and orange-colored cottonseed pigments, gossypurpurin (2) and gossyfulvin (4), are, likewise, polyphenolic and are derivatives of gossypol.

Cottonseed pigments are unique not only in their chemical nature but also in their manner of occurrence in the seed. In summarizing the distribution of the more important classes of plant pigments, HAAS and HILL (10) noted that they usually occur in one of three ways: in solution in the cell sap, as in the case of most anthocyanins; as powdery deposits in the bark or on the leaves, stems, flowers, and seed capsules, as in the case of many flavones; or in the plastids, as in the case of the carotenoids and chlorophylls. Most of the pigments of cottonseed are contained in distinct morphological structures, which are relatively large ovoid or spherical bodies, 100–400 μ on the long axis, and have been variously designated as "secretion cavities," "resin glands," "oil glands," or "gossypol glands" (31). Pigment glands are characteristic of all species of *Gossypium* and seem to be peculiar to this genus and to a very few closely related genera of the subfamily Hibisceae (26, 31).

The earliest investigators of the structure of the cottonseed (6, 12, 13) noted the occurrence of pigment glands and reported the existence of a gland "membrane" made up of two layers, the outer layer consisting of thin-walled, tangentially flattened cells and the inner layer of mucilaginized material in which traces of cell walls were still evident. HANAUSEK (12, 13) noted that successive treatment of the gland with water and solutions of hydrochloric acid and potassium hydroxide revealed the mucilaginized layer as a yellow, folded, and laminated mass. Subsequent investigators have neither added to the early descriptions of the gland "membrane" nor confirmed its existence.

The pigment glands are very resistant to mechanical rupture as well as to the action of a number of organic liquids; and, when cottonseed kernels are comminuted while in contact with a mixture of inert organic solvents (density 1.378 gm./ml.), the intact glands will rise to the surface, with the other seed tissue falling to the bottom (5). Although the assumption that the pigments are confined within a resistant membrane seemed to constitute the most logical explanation of their behavior, it might be argued that they occur in peculiarly resistant agglomerations held together by surface forces, as has been suggested in the case of plant plastids (32).

The present investigation was undertaken with the object of obtaining further information concerning the structure and properties of the pigment glands. All experiments were carried out with samples of three pure-bred strains of *Gossypium hirsutum*.

Observations

Pigment glands are profusely distributed over the cotyledons and hypocotyls and are absent only from the lower part of the radicle of the cottonseed embryo. Their relatively large size, as well as their distribution, is shown in figure 1, which represents a free-hand section of a seed.

Microscopic examination confirmed the observations of HANAUSEK (13) and VON BRETFELD (6) that contact with water induces immediate rupture of the glands. Rupture occurs within a fraction of a second after immersion, and the contents flow out in vigorously moving streams of minutely divided, suspended particles in lively motion. After the gland is emptied, a nearly transparent structure remains which retains the shape of the original gland. This series of changes is shown in figure 2. In some cases the "streaming" is spasmodic, at first very vigorous, slowing down after a short time, and later renewing its original intensity. In a few cases as many as four simultaneous streams of suspended material flowed from a single gland.

The reaction of separated glands was the same regardless of the means employed for their separation—that is, whether separated manually or by the flotation process. Glands allowed to remain in contact with the surrounding tissue and oil on the surfaces of freshly cut cross sections of cottonseed reacted with water in a fashion entirely analogous to that of separated glands. Preliminary defatting of ground or flaked cottonseed did not alter the reaction of intact glands with water.

PIGMENT GLAND WALL

STRUCTURE.—In order to investigate the structure of the emptied gland, individual glands were soaked overnight in alcoholic acetic acid (5% acetic acid in 95% ethyl alcohol) and then in 25% aqueous ammonium hydroxide. They were then mounted in water for microscopic examination. This treatment freed the glands of their contents and removed particles adhering to the outside. Gland walls so prepared are observed to be composed of five to eight, relatively thick and almost colorless, curved plates of irregular shape, fitted together in a pattern somewhat resembling the cover of a baseball (fig. 3). An unusual property is manifested in the marked tendency of the plates of the emptied glands to cohere in their original configuration even after the prolonged treatment described above.

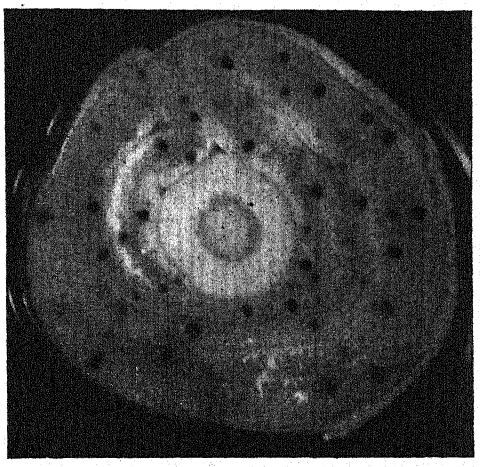


FIG. 1.—Cross section of cottonseed through radicle and cotyledons, showing distribution of pigment glands. ×14. (Photomicrograph by T. L. W. BAILEY.)

The plate-like structure of the walls is characteristic not only of separated glands but also of glands in the seed. The curved and bulging outlines of the plates may be readily observed in glands in seed sections by changing the focus of the microscope. In the case of glands separated by flotation and subsequently sieved in a dry state to remove particles of defatted meal, the outlines of the plates are particularly evident because of the packing of finely divided defatted

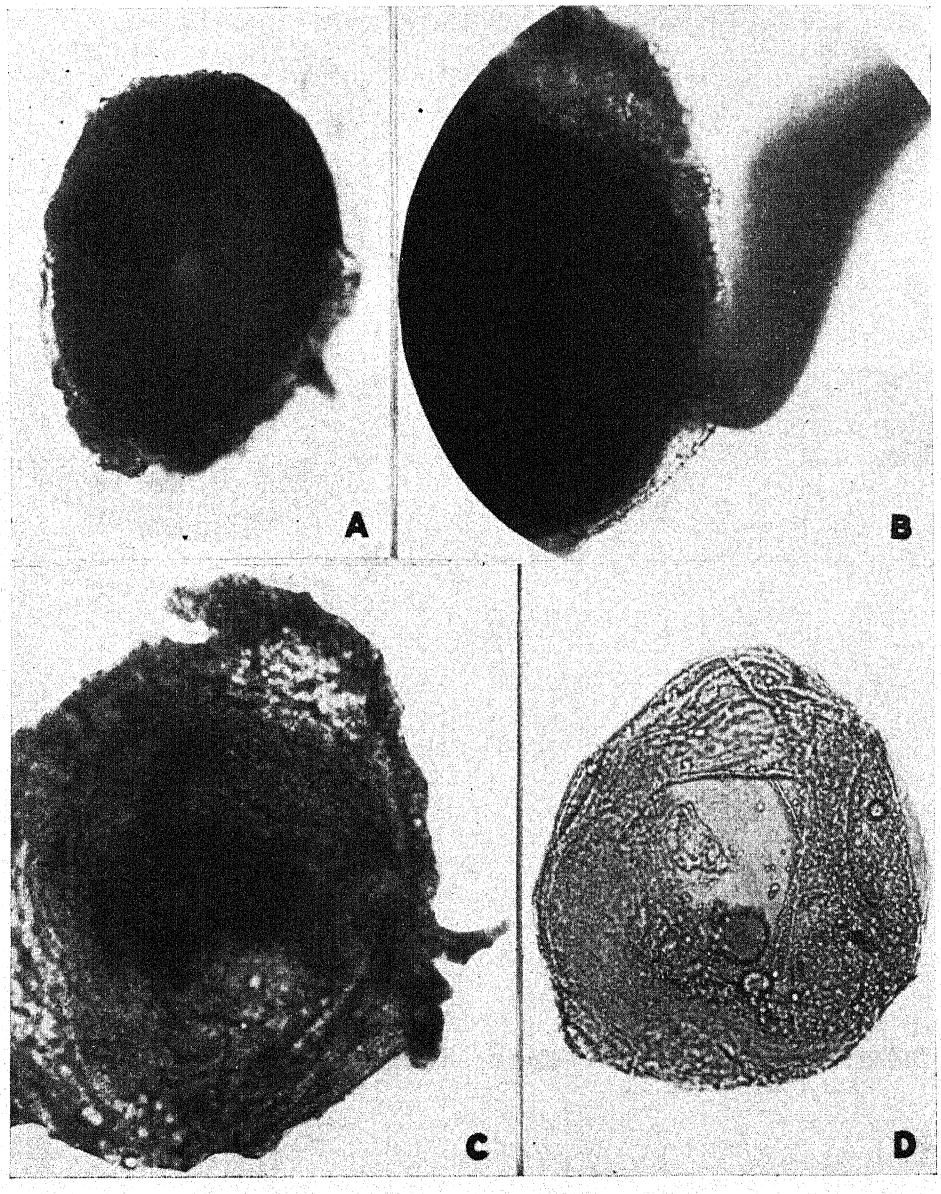


FIG. 2.—Single cottonseed pigment gland reacting with water: *A*, beginning of rupture; *B*, contents emptying; *C*, almost emptied; *D*, completely emptied. ×500. (Photomicrographs by M. L. ROLLINS.)

meal particles along the seams between the plates.

The action of water alone is entirely analogous to that of aqueous reagents. Figure 2*D* shows that, despite the vigorous reaction involved in the discharge of the pigments during immersion in water, only the front plate of the gland has been removed, and the other plates have remained joined together as in the original intact gland.

observed in the ruptured glands. Portions of gland walls consisting of several adjoined plates have the appearance of empty shells floating in the liquid of the mounting medium, and crushing them reveals no inner contents. Closer observation of what at first appears to be a circular structure in the center of each plate reveals it to be merely a thickened area which probably accounts for the curvature of the plates. The striations

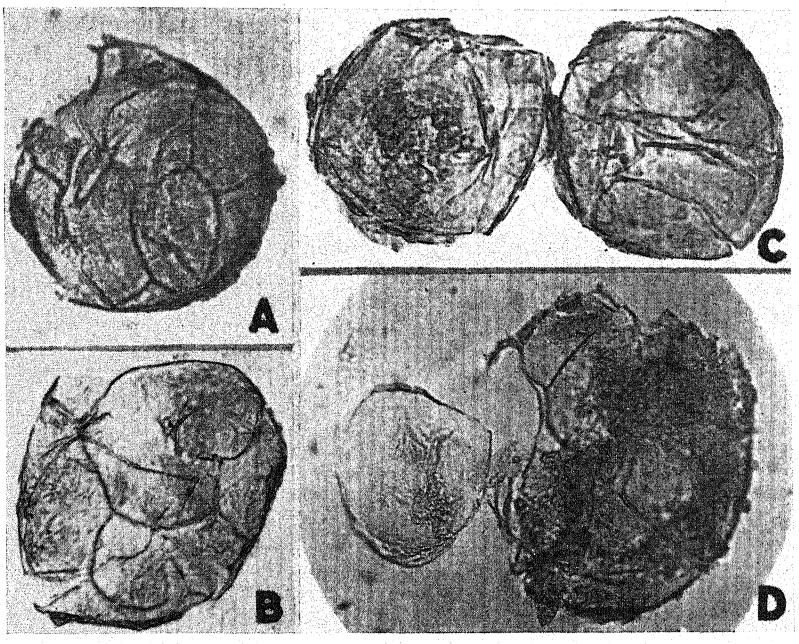


FIG. 3.—Emptied cottonseed pigment glands showing wall structure. ×250. (Photomicrographs by M. L. ROLLINS.)

On the basis of striations observed in sections of cottonseed pigment glands, previous investigators (31) have reported the existence of residual cell walls in the interior. The mechanical strength of the glands and the tendency of the plates to remain in position after the glands are emptied, as well as the spasmodic nature of the emptying process, also suggested the existence of secondary or internal walls. During the present investigation, however, no internal structure could be reported by STANFORD and VIEHOEVER (31) are readily observed in sections of the glands (fig. 4). However, since examination of a large number of cross sections reveals no regular arrangement of the striations, it can be concluded that they are merely fissures produced by mechanical pressure on the gelatinous contents of the glands.

CHEMICAL COMPOSITION.—Microchemical investigation of the nature of cleaned and emptied glands yielded the results

reported in table 1. Conclusions concerning the chemical nature of the constituents of the walls were based on the properties described by HAAS and HILL (10).

The absence of proteins was indicated by the failure of the emptied gland to react with Millon's reagent; the absence of lignin and pentosans, by lack of reaction with phloroglucin and hydrochloric acid.[1]

Staining of the outer surface of water-washed gland walls with Sudan IV indicated a coating which might be suberin, cutin, or wax. Since the reaction with Sudan IV was not affected by prior washing of the glands with petroleum naphtha, it was concluded that little, if any, wax occurred in the coating. A further indication of the presence of suberin and cutin was the yellow stain obtained with concentrated potassium hydroxide. A negative test with iodine–zinc chloride after heating showed that the compound was cutin rather than suberin.

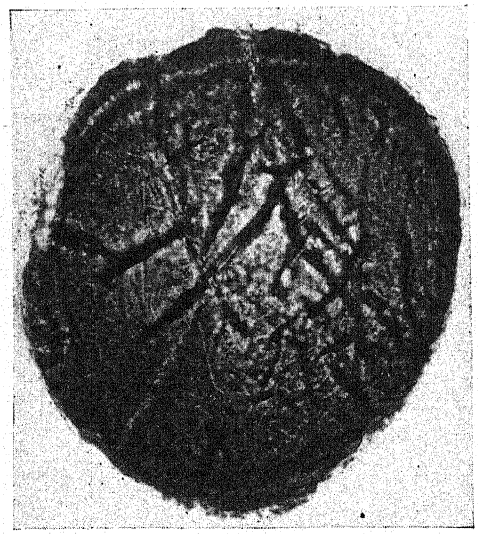


FIG. 4.—Cross section of cottonseed pigment gland showing striations. ×440. (Photomicrograph by T. L. W. BAILEY and I. V. DEGRUY.)

Emptied glands stained with Sudan IV were mottled in appearance. Observation of the distribution of Sudan IV applied to sections of emptied glands in seed sections showed that cutin occurs as an uneven, continuous, outer coating of the glands. After the presence of cutin had been established by staining reactions, it was possible to obtain further information concerning its distribution on the gland walls. Microscopic examination of intact glands, without application of stains, showed a relatively thick transparent layer of material in the region of the centers of the plates composing the gland walls and a thin layer at the edges of the plates, so that the intact glands are very irregular in outline. In view of the previously described staining reactions, this thick layer is probably cutin.

The cutin was completely removed from glands which had been immersed in alcoholic acetic acid for 24 hours and then in aqueous ammonium hydroxide for 24 hours. Thus, as with cutin in the skin of apples (23), it appears probable that the cutin is not chemically combined with the other wall constituents but forms a separate layer or incrustation around the glands.

The positive reactions obtained with diagnostic cellulose stains indicated that cellulose is the principal wall constituent, but the modified color observed suggested the presence of other substances. Treatment of the walls with ammoniacal cupric hydroxide resulted in their partial solution. However, the residual material retained the original configuration so that it had the appearance of "ghosts" of

[1] Recent microchemical observations of the reaction of cottonseed with phloroglucin-hydrochloric acid and other pentosan reagents (28, 29, 31) showed the reaction to be localized in the pigment glands. The present observation that pentosans or pentosan-like substances do not occur in the walls confirms the further observation (9) that these compounds constitute part of the gland contents.

the plates composing the walls. Consequently, it was concluded that the cellulose is impregnated with the materials responsible for the modified colors obtained with cellulose stains.

Efforts to determine the nature of the material with which the cellulose was impregnated were not conclusive. The original water-washed glands, as well as those treated with cupric ammonium hydroxide, developed a red-purple color with ruthenium red which suggested the presence of pectins, hemicelluloses (14), or other uronic acid derivatives. Boiling the walls in 0.5% ammonium oxalate to remove pectins left "ghosts" which still stained with ruthenium red. After emptied glands had been treated with dilute sodium hydroxide, in which hemicelluloses are soluble, the residual "ghosts" likewise gave a positive reaction with ruthenium red. Moreover, following successive treatments with dilute aqueous hydrochloric acid, ammonium hydroxide, and sodium hydroxide, to remove both pectin and hemicellulose, the "ghosts" of the plates still stained with ruthenium red. Thus it appears that very stable and insoluble uronic acid derivatives other than pectin and hemicellulose form part of the gland walls. The failure of the emptied glands to react with phloroglucin and hydrochloric acid demonstrates the unusual stability in the gland walls of the materials which stain with ruthenium red.

TABLE 1

MICROCHEMICAL REACTIONS OF PIGMENT GLAND WALL

Preparation of glands*	Reagent	Observation	Conclusions
.....	Millon's reagent	No reaction	Protein absent
.....	Phloroglucin and hydrochloric acid	No reaction	Lignin and pentosan absent
.....	Sudan IV	Mottled red stain	Wax, cutin, or suberin present
Hexane washed before emptying.....	Sudan IV	Mottled red stain	Cutin or suberin present
.....	Conc. potassium hydroxide	Yellow stain	Cutin or suberin present
.....	Conc. potassium hydroxide; heat	Yellow stain	Cutin or suberin present
Heated with conc. potassium hydroxide.....	Iodine–zinc chloride	Yellow stain	Cutin present
	Sudan IV	No stain	Cutin removed
	Delafield's haematoxylin	Purple stain	Cellulose present
	Iodine green	Dark gray-green stain	Impregnated cellulose present
Alcoholic acetic acid; aqueous ammonium hydroxide.....	Iodine-potassium iodide	Yellow stain	Impregnated cellulose present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose present
	Congo red	Yellow-orange stain	Impregnated cellulose present
	Ammoniacal cupric hydroxide	Partial solution	Cellulose and hemicellulose dissolved
Ammoniacal cupric hydroxide.....	Ruthenium red	Red stain	Uronic acid derivatives present
.....	0.5% ammonium oxalate; heated	Partial solution	Pectin removed
Boiled in ammonium oxalate.....	Ruthenium red	Red stain	Uronic acid derivatives present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose
.....	Dilute sodium hydroxide	Partial solution	Hemicellulose present
Dilute sodium hydroxide.	Ruthenium red	Red stain	Uronic acid derivatives present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose

* The glands were first treated with water to empty them of pigments.

BEHAVIOR OF PIGMENT GLANDS TOWARD VARIOUS LIQUIDS

In a previous investigation (5) microscopic observations of the effect of a number of organic liquids on cottonseed pigment glands had shown the liquids to fall into two categories with respect to their activity toward the glands: those which had little or no effect even after 24 hours of contact and those which caused the glands to lose their contents after contact for 24 hours or less. The "inert" group included hydrocarbons, such as *n*-hexane, mineral oil, benzene, and tetralin; chlorinated hydrocarbons such as carbon tetrachloride, 1,1,2-trichloroethane, 1,1,2-trichlorethylene, 1,1,2,2-tetrachloroethane, and 1,1,2,2-tetrachlorethylene; and triglycerides such as cottonseed oil. The "active" group included polar liquids of low molecular weight, such as acetone, methyl ethyl ketone, 1,4-dioxane, diethyl ether, and chloroform.

When mixtures of "inert" liquids were used for the mechanical separation of glands from other cottonseed tissue by the flotation process (5), the coloring observed in the liquid mixtures indicated some rupture of the glands. It was found that the number of glands ruptured could be greatly reduced by shortening the time of exposure to the liquids and by carrying out the separation at low temperatures. With the establishment of the existence and properties of the pigment gland wall, it seemed probable that further investigation might reveal the cause of the differences in the reaction of the glands toward various liquids.

Closer observation of the effect of water on glands revealed that the reaction occurs in several successive stages. Immediately following contact with water the contents of the gland, which are originally perfectly clear, become cloudy and lighter in color. Immediately thereafter the pigments are expelled through one or more fissures between the plates in rapidly moving streams of finely divided, suspended particles exhibiting lively Brownian movement.

In view of the insolubility of the pigments in water, the preliminary clouding of the gland contents demonstrates that water enters the glands prior to the expulsion of its contents.

The explosiveness with which the glands empty suggests that rupture might occur as the result of pressure built up by the diffusion of water into the glands through the walls. However, the effects of various saturated aqueous solutions on the glands does not support this conclusion. The glands burst almost instantly upon contact with saturated aqueous solutions of dextrin, sucrose, glucose, urea, sodium chloride, ammonium sulfate, sodium acetate, and calcium chloride. Modification of the pH of the solutions by the addition of acid or base did not affect the speed with which the glands ruptured. They burst rapidly upon immersion in glycerine containing 10% water. In pure glycerine they were not immediately affected, but each gland burst at several points after prolonged contact, and sluggishly moving streams of suspended material emanated from the surface of the gland.

Microscopic observation of the behavior of the glands in various water-miscible anhydrous organic liquids revealed that all these liquids extract the pigments from the glands but that the reaction is very much slower than with water (table 2). After contact with anhydrous methanol, ethanol, acetone, methyl ethyl ketone, or 1,4-dioxane for an hour, the glands were completely emptied. The mechanism of the reaction could not be observed because of the solubility of the

pigments in these solvents. Isopropanol had very little effect on the glands even after prolonged contact. As shown in table 2, the glands rupture more rapidly upon contact with mixtures of these organic liquids and water, and the speed of rupture increases in direct proportion to the amount of water contained in the mixture. It can be concluded that the gland walls are somewhat susceptible to the action of these polar organic molecules of low molecular weight but are much more susceptible to the action of water.

In view of these observations, it seemed probable that organic liquids, such as chloroform and diethyl ether, were themselves incapable of affecting the pigment glands; that their previously observed action resulted from the small amounts of water dissolved in them; and, furthermore, that the slowness with which these liquids extract the pigments from the glands can be attributed to their

TABLE 2

EFFECT OF SOLVENTS ON COTTONSEED PIGMENT GLANDS

Solvent	Water content (%)	Observed reaction of glands
Methanol	0	None ruptured; yellow extract darkening upon longer contact
	5	Small fraction ruptured after 15 minutes; very short purple streams* and yellow extract subsequently darkening
	10	Small fraction ruptured after 10 minutes; very slowly moving streams
	25	Most ruptured after 5 minutes; slowly moving streams
	50	Almost all ruptured immediately; rapidly moving streams
Ethanol	0	None ruptured; yellow extract darkening upon longer contact
	5	Small fraction ruptured after 20 minutes; pale-yellow extract subsequently darkening
	10	Small fraction ruptured after 10 minutes; pale-yellow extract subsequently darkening
	20	Most ruptured after 2–3 minutes; very short sluggish streams; yellow extract subsequently darkening
	50	Most immediately ruptured; rapidly moving streams; yellow precipitate
Acetone	0	Most ruptured after 10 minutes; yellow extract darkening upon longer contact
	5	Most ruptured after 3–4 minutes; yellow extract subsequently darkening
	10	Most ruptured after 2–3 minutes; yellow extract with purple precipitate
	25	Most ruptured after 1–2 minutes; yellow extract with purple precipitate
	50	Most ruptured immediately; rapidly moving streams
Dioxane	0	None ruptured; pale-yellow extract darkening upon longer contact
	10	Most ruptured after 4–5 minutes; pale-yellow extract subsequently darkening
	25	Most ruptured after 2–3 minutes; very few streams
	50	Most ruptured almost immediately; rapidly moving streams
Isopropanol	0	None ruptured; pale-yellow extract darkening upon longer contact
	5	Very small fraction ruptured after 30 minutes; yellow extract subsequently darkening
	10	Small fraction ruptured after 8 minutes; yellow extract subsequently darkening
	20	Most ruptured after 4 minutes; yellow extract subsequently darkening
	50	Most ruptured after 2–3 minutes; yellow extract subsequently darkening
	60	Most ruptured immediately; rapidly moving streams

* Streams designate finely divided suspended particles in lively motion emanating from pigment glands.

slight water content. In confirmation of this hypothesis, it was found that, whereas undried ether or chloroform extracts all the pigments[2] from the glands after contact for 24 hours (3), under the same conditions ether or chloroform, previously dried over calcium chloride or sodium sulfate, extracts only a small fraction of the pigments from either dried, separated glands or dried, finely ground cottonseed. On the other hand, when dried glands are first macerated with sharp sand so that the walls are broken, the pigment contents are instantly soluble in either dry ether or dry chloroform.

The action of commercial-grade organic liquids, such as ether or chloroform containing traces of moisture, may be postulated as follows: the water, because of its low concentration in these solvents, ruptures the gland walls very slowly; the organic liquids enter the glands through the ruptures and dissolve their contents; and the pigment solutions then slowly diffuse out of the glands through the ruptured walls. It was observed, in support of this view, that when glands were immersed in commercial-grade chloroform and the chloroform was allowed to evaporate after several hours contact, many of the glands were still intact, but some were ruptured, with mixed crystals of the pigments emanating from the interiors through the ruptures in the walls.

FORMATION AND PROBABLE FUNCTION OF PIGMENT GLANDS

It has been reported that the first sign of the presence of pigment glands can be detected in the 15-day-old embryo, and by the 16th day the glands are as fully developed structurally as in the mature seed (27, 31). Prior to the formation of the pigment gland, a well-defined circle of cells marking the boundary of the developing gland can be noted. These cells have been assumed to constitute an integral part of the gland because of their persistence in the mature seed (13, 27, 29, 31). It is evident that this cannot be true in view of the fact that intact glands separated from the seed are free of these cells. On the other hand, the formation of the water-sensitive gland wall in the moist surroundings of the developing seed might logically be attributed to the protective action of the encircling layer of cells, which always forms before the pigment gland develops and probably functions as a protective sheath (11).

Gossypol cannot be detected in the embryo until the 15th–18th days (7, 27). After its first appearance, its concentration increases many fold and at a rate greater than that of any of the other components of the seed (7). The sequence of gland formation followed by synthesis of gossypol suggests that this very unstable polyphenolic pigment cannot be formed until it is furnished with a protected region for its development and that the subsequent rapid formation of gossypol within the gland is the result of the protective action of the gland walls.

It has been reported that the pigments of dry seed undergo relatively little change during storage (25), whereas seed of high moisture content store less well than dry seed (16, 21, 30) and yield more deeply colored oils (19, 20). It seems probable that many of the transformations occurring during the storage of moist seed can be attributed to the inadequate protective action of the sheath

[2] The gossypol content of the extracts was determined by means of the gossypol-antimony trichloride spectrophotometric method (3); and their gossypurpurin content was estimated on the basis of the height of the absorption band at 570 A characteristic of gossypurpurin (2).

of cells surrounding the pigment glands. In the presence of excessive amounts of moisture, for example, when a thick seed section is treated with several drops of water, the gland contents gradually become cloudy, indicating the entrance of moisture through the surrounding tissue. Although no clouding can be observed in the glands of seed which have been stored at such high moisture levels as to exhibit significant color deterioration in the oil, it seems probable that moisture has entered the glands in amount sufficient to affect their contents. Furthermore, the observation that increased moisture accelerates the respiration rate of stored cottonseed (17, 20) suggests that the polyphenolic gland pigments, like other naturally occurring phenols (24), participate as respiratory intermediates in the metabolism of the seed. On the basis of these combined observations it seems probable that the deeper color of the oils obtained from seed stored at higher than at optimum moisture levels can be attributed to the more active participation of the gland pigments in the respiration of the seed, and their conversion thereby into deeply colored quinoid oxidation products. These reactions would produce an increased intensity of respiration and would result in increased color in the oil.

Summary

1. The cottonseed pigment gland has been shown to possess a rigid, thick wall inclosing a gelatinous suspension of the gland pigments.

2. The gland wall consists of five to eight irregularly shaped, curved plates, which appear to be composed of cellulose impregnated with pectin, hemicellulose, and other unidentified uronic acid derivatives. The glands are heavily incrusted with cutin.

3. The pigment glands have been shown to be resistant to the action of most reagents except water and polar organic liquids of low molecular weight. Their reaction to water consists in the immediate rupture of the wall along one or more of the seams between the plates, followed by expulsion of the gland contents as finely divided suspended particles. Water-miscible, polar organic liquids of low molecular weight affect the glands very much more slowly than water; and the extremely slow action of other organic liquids has been shown to be attributable to their slight moisture content.

4. The existence of a highly resistant pigment gland wall has been proposed to explain the anomaly of the relative stability of reactive polyphenolic pigments in cottonseed. The sensitivity of the glands to moisture has been proposed to explain various changes observed during maturation of cottonseed as well as changes which occur during the storage of moist cottonseed.

The authors wish to express their appreciation of the generosity of W. R. PADEN and J. WINSTON NEELY in supplying the samples of cottonseed with which this investigation was carried out.

SOUTHERN REGIONAL RESEARCH LABORATORY
NEW ORLEANS, LOUISIANA

LITERATURE CITED

1. ADAMS, ROGER; GEISSMANN, T. A.; BUTTERBAUGH, D. J.; and KIRKPATRICK, E. C. Structure of gossypol. XV. An interpretation of its reactions. Jour. Amer. Chem. Soc. 60:2193–2204. 1938.

2. BOATNER, C. H. The pigments of cottonseed. Oil & Soap 21:10–15. 1944.

3. BOATNER, C. H.; CARAVELLA, M.; and KYAME, L., Quantitative determination of extractable gossypol in cottonseed and cottonseed meal. A

spectrophotometric method. Ind. Eng. Chem. Anal. Ed. 16:566–572. 1944.
4. BOATNER, C. H.; CARAVELLA, M.; and SAMUELS, C. S. An orange-colored pigment of cottonseed. Jour. Amer. Chem. Soc. 66:838. 1944.
5. BOATNER, C. H., and HALL, C. M. The pigment glands of cottonseed. I. Behavior of the glands toward organic solvents. Oil & Soap 23:123–128. 1946.
6. BRETFELD, VON. Anatomie des Baumvolle- und Kapoksamens. Jour. Landw. 35:29–56. 1887.
7. CASKY, C., JR., and GALLUP, W. D. Changes in the sugar, oil, and gossypol content of the developing cotton boll. Jour. Agr. Res. 42:671–673. 1931.
8. CLARK, E. P. Studies on gossypol. I. The preparation, purification, and some of the properties of gossypol, the toxic principle of cottonseed. Jour. Biol. Chem. 75:725–739. 1927.
9. GUREVICH, M. Anatomic-michrochemical investigations of the seed of two trans-Caucasian sorts of cotton-plant. Symposium by Union Institute for Scientific Research on Fats. Pp. 31–42. 1936.
10. HAAS, P., and HILL, T. G. An introduction to the chemistry of plant products. Vol. 1. 4th ed. Longmans, Green & Co., New York. 1928.
11. HABERLANDT, G. Physiological plant anatomy. 4th ed. Trans. M. DRUMMOND from the German. Macmillan, New York. 1914.
12. HANAUSEK, T. F. Pp. 754–759 *in* J. WIESNER, Die Rohstoffe der Pflanzenreiches, Vol. 2. 2d ed. Leipzig. 1903.
13. ———. Microscopy of technical products. Trans. A. L. WINTON from the German. Wiley, New York. 1907.
14. HAWLEY, L. F. The differentiation of hemicelluloses. Ind. Eng. Chem. 24:1190–1194. 1932.
15. HAWORTH, R. D. Natural naphthaquinone pigments. Ann. Rep. Chem. Soc. 36:284–286. 1939.
16. KARON, M. L., and ALTSCHUL, A. M. Effect of moisture and of treatment with acid and alkali on rate of formation of free fatty acids in stored cottonseed. Plant Physiol. 19:310–325. 1944.
17. ———, and ———. The respiration of cottonseed. Plant Physiol. (In press.)
18 KARRER, P., and TOBLER, E. Pflanzenfarbstoffe, 44, Einige Beobachtungen über Umsetzungen des Gossypols. Helv. Chim. Acta. 15:1204–1212. 1932.
19. KYAME, L., and ALTSCHUL, A. M. Comparison between respiration, free fatty acid formation, and changes in spectrum of seed oil during the storage of cottonseed. Plant Physiol. (In press.)
20. MALOWAN, J. Changes in cottonseed during heat and storage. Cotton Oil Press 5:40–43. 1921.
21. ———. How cottonseed deteriorates through moisture. Oil and Fat Ind. 4:127–130. 1927.
22. MARCHLEWSKI, L. Gossypol, ein Bestandteil der Baumvollsamens. Jour. prakt. Chem. 60: 84–90. 1899.
23. MARKLEY, K. S., and SANDO, C. E. Progressive changes in the cuticle of apples during growth and storage. Jour. Agr. Res. 46:403–412. 1933.
24. NELSON, J. M., and DAWSON, C. R. Tyrosinase. Adv. Enz. 4:99–152. 1944.
25. PODOL'SKAYA, M. The transformation of gossypol in the ripening and preservation of the cotton plant. Masloboina Zhirovoe Delo 15:9–10. 1939. (Chem. Abst. 34:1955. 1940.)
26. REEVES, R. G. Comparative anatomy of the seeds of cotton and other malvaceous plants. II. Hibisceae. Amer. Jour. Bot. 23:394–405. 1936.
27. REEVES, R. G., and BEASLEY, J. O. The development of the cotton embryo. Jour. Agr. Res. 51:935–944. 1935.
28. ———, and ———. Localization of pentosans in the resin glands of the cotton embryo. Jour. Agr. Res. 54:711–718. 1937.
29. REEVES, R. G., and VALLE, C. C. Anatomy and microchemistry of the cotton seed. BOT. GAZ. 93:259–277. 1932.
30. ROBERTSON, F. R. and CAMPBELL, J. G. Some observations on the increase of free fatty acid in cottonseed. Oil & Soap 10:146–147. 1933.
31. STANFORD, E. E., and VIEHOEVER, A. Chemistry and histology of the glands of the cotton plant, with notes on the occurrence of similar glands in related plants. Jour. Agr. Res. 13:419–437. 1918.
32. WEIER, T. E. The structure of the chloroplast. Bot. Rev. 4:497–530. 1938.

PHOTOMICROGRAPHIC COMPARISON OF MILKWEED BAST AND COTTON LINT IN RELATION TO CELL-WALL STRUCTURE OF OTHER NATURAL CELLULOSE FIBERS

MARY L. ROLLINS[1]

Introduction

In competition with cotton in various phases of commercial utilization are the bast fibers of flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), jute (*Chorchorus capsularis*), and ramie (*Boehmeria nivea*). Numerous other bast fibers of minor commercial importance rose to prominence during the wartime fiber scarcity, and some new to commerce were proposed as substitute fibers. Among the latter the giant milkweed (*Asclepias syriaca* [15, 26]), said to have been used as cordage fiber by various primitive peoples, was suggested as suitable for exploitation.

In the course of preliminary microscopical investigations to determine the possibilities of the bast fiber of milkweed, the incidental observation was made of a surprisingly close parallel structure between the seed hair of cotton and the bast fiber of the milkweed stem. As a consequence, the present investigation was undertaken to illustrate the morphological similarity between this bast fiber and cotton as a basic principle of nature in the structural design of cell walls of the fibrous type.

Accordingly, the two widely different types of fibers were compared under the microscope and copiously photographed. To facilitate the study of cell-wall structure, specific swelling techniques, based on the solvation of cellulose in certain reagents, were applied. The milkweed fiber, having the advantage of being relatively large, with few cell-wall layers, lends itself particularly well to microscopic study and photomicrography of cell-wall details; and photomicrographs of this fiber are of considerable aid in the interpretation of the finer and more intricate structure of the seed hair of cotton which is less easily seen.

Admittedly, none of the pictures represents the true condition of fiber cell walls in the living plant, since the chemical treatments necessary to induce sufficient swelling to reveal the inner fiber structure of the dead and desiccated cells undoubtedly alter their natural arrangement. That the fiber elements revealed in this investigation are not all artifacts, however, is supported by ample evidence scattered through the literature of a similar pattern of cell-wall structure existing in other cells of the fiber type, especially those that have gained commercial importance because of their natural strength.

Review of literature

The literature of fiber structure is voluminous, covering at least a hundred years of microscopical study. Extensive reviews of the early work were given by VAN ITERSON (34, 35), and a summary of the more recent investigations is found in chapter iii, C, of *Cellulose and Cellulose Derivatives* (29). This present paper is concerned chiefly with the review of the photographic record.

[1] Southern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U.S. Department of Agriculture.

STRUCTURE OF COTTON FIBER

The cotton fiber is a single-celled seed hair, growing out from an epidermal cell of the seed coat. Also arising from the seed coat, about 6 days after the true lint fibers have emerged, are short coarse fibers known as linters or fuzz fibers. These differ from the true lint fibers in that they vary in color, whereas the true lint is generally white. In addition, they are usually coarse and thick-walled and, when examined at a low magnification, are shown to have a number of twisted, enlarged abnormalities. Because of its commercial significance the cotton fiber has been the subject of the most extensive microscopical investigations, and considerable evidence has been gathered as to the mechanism of its growth and the details of both microscopic and submicroscopic structure.

BALLS (7), in 1919, showed that concentric layering becomes visible in the swollen walls of cotton fibers treated with sodium hydroxide and carbon bisulfide. It was found that there is only one thin primary layer while the hair is growing in length but that, as soon as thickening of the wall sets in, the number of layers increases day by day up to a maximum of twenty-five when thickening is complete. BALLS thought that, since growth was arrested by sunshine in the middle of each day in Egypt, the number of these layers corresponded to the number of nights during which the growth in thickness of the cell wall had continued. These concentric layers were thus considered to be rings of nightly growth, differentiated by heterogeneity like the annual rings in timber. These rings are coarser, more sharply demarcated, and less regular in fuzz hairs than in lint hairs, but the later rings in fuzz hairs may resemble those of lint hairs.

BALLS and HANCOCK (8), in 1921, found that every growth layer of the cell wall had a spiral structure and that the pattern of the spiral which appears to be predetermined during growth in length is preserved through all the growth rings of the secondary-wall thickening. They advanced the further theory that the pattern (direction, reversal, and pitch) of these spirals seems to be the major determinant of the externally visible convolutions of the hair.

DENHAM (12), in 1922, followed this work further, indicating that, in striations which occur in all parts of the hair and in all layers of the wall, the direction followed is not always the same in superimposed layers. He corroborated BALLS's idea that the convolutions follow the direction of the primary-wall striations and stated that the cause of the convolutions is a double spiral line of weakness. Excellent photomicrographs were shown of the so-called "slip planes" in the external layer of the fiber.

In 1937 KERR (20) investigated the structure of growth rings in the secondary wall of the cotton hair. In contradiction to BALLS and HANCOCK, who had pictured each growth ring as a separate cylinder or shell of cellulose, KERR concluded that the secondary wall is a complex and coherent matrix and that the appearance of layering in swollen fibers results from a structural differentiation of the cellulose into alternating bands of compact, strongly birefringent zones with more porous, comparatively isotropic lamellae. He found also that during the period of secondary-wall deposition two lamellae, one compact and one more porous, are deposited every 24 hours, and that these two lamellae constitute a daily growth ring. From microscopical observations in which stains were used, it was assumed that compact zones of growth rings are deposited dur-

ing the day and the porous zones at night. Evidence was offered that variations in density are correlated with fluctuation in temperature, the more porous areas being deposited in periods when the temperature dropped below 20° C.

The following year (1938) ANDERSON and KERR (4) showed, in work reported on cotton fibers in various stages of development, that the cell diameter is reached soon after the hair originates but that elongation continues for from 15 to 20 days. Their conclusions as to fiber structure of the seed hair of cotton were summed up as follows:

> The cell wall is composed of
>
> 1. A primary wall containing cellulose and pectic substances.
>
> The cellulose micelles in this wall are grouped into delicate anastomosing threads which have at least two systems of orientations:
>
> (*a*) a flat right-hand spiral, (*b*) a flat left-hand spiral and probably also (*c*) a transverse position. All three systems seem uniform over the entire surface of the fiber cell.
>
> 2. A secondary wall composed of many lamellae of cellulose. The lamellae are not separated from one another by noncellulosic substances but represent dense and less dense areas of cellulose. The layers are formed of systems of spirally wound branching threads and the direction of the spiral is reversed at frequent intervals.
>
> 3. Frequently the pattern of spirals first appearing in the secondary wall is not similar to that in subsequent layers of the wall. Most of the layers of the wall, however, follow a pattern that is established soon after secondary thickening has begun.[2]

In 1941 HOCK, RAMSAY, and HARRIS (18) corroborated much of the previous work of ANDERSON and KERR. They found the cell wall of cotton fiber to consist of a primary and secondary wall. The latter comprises the bulk of the fiber and is made up of innumerable spirally oriented cellulose fibrils inclosed by a winding which also makes a spiral, but in the opposite direction from the former. Both winding and fibrils reverse their direction at frequent intervals along the axis of the fiber, their points of reversal being coincident. The secondary wall is inclosed by a thin primary wall made up of fine crisscrossing strands of cellulose, imbedded in a membrane consisting principally of wax and pectic substance. They stated that, when cotton fibers are swollen under certain conditions, a lamellate structure is discernible in the secondary wall and that the number of these lamellae increases with the age of the fiber.

In 1946 KERR (21) published photomicrographs to show the distinction between the primary-wall membrane and the first ("winding") layer of the secondary wall in undried mature fibers. He stated that in swelling both layers behave so as to restrict the expansion of the cell-wall thickening.

STRUCTURE OF FIBERS OTHER THAN COTTON

Among the bast fibers which have been investigated microscopically for structure, flax has received the most intensive study. In 1922 NODDER (27) published an account which included drawings of the microscopical features of flax, jute, and hemp and photomicrographs of flax, ramie, and hemp. The fibers had previously been swelled with strong solutions of calcium chloride and disintegrated by pressure on the cover slip of the slide. The concentric layering of the walls of these fibers and the spiral arrangement of "fibrils" of which they are composed were clearly shown.

ANDERSON (3), in 1927, studied flax fibers by swelling in 50% sulfuric acid and iodine, and the internal structures were made visible by pressure on the

[2] Quoted with the permission of the copyright owner, the American Chemical Society.

coverslip. His photomicrographs show, first, that ballooning occurs in the early stages of the swelling because of constriction owing to failure of the outer sheath to swell; second, that the various lamellae of the wall may be slipped from one another; and, third, that the outer sheath appears to be made of rather wide spirals. In speaking of the layers of the cell wall, ANDERSON rejected the conception that the dark lines between the layers are made of actual material but stated that these lines merely mark the boundaries between successive lamellae.

In 1942 HOCK (17), in a paper on the microscopic structure of flax as compared with the other bast fibers, presented photomicrographs of cross sections and longitudinal views of the fibers swollen in cuprammonium hydroxide to show the lamellate nature of the cell wall, the spiral nature of fibrillae, and the "ballooning" resulting from constrictions of the outermost layers of fibrillae. He stated that the fibrillae of the outermost layer of the secondary wall spiral in a direction opposite from that of the fibrillae in all the other layers of the secondary wall. He attributed to ramie and flax an identical fibrillate structure, namely, an S twist on all layers except the outermost layer, the fibrils of which have a Z twist. In hemp and jute, on the other hand, he found that, although the fibrils of the outermost layer have the Z twist, the fibrils of the inner layers lie almost parallel to the axis of the fiber. His photomicrographs of flax fibers show concentric rings in swollen cross sections, parallel lamellae in longitudinal view, the actual separation of wall layers by excessive swelling, and the ballooning caused by constrictions of the unswollen outer wall.

Structures similar to those shown in cotton and flax have been demonstrated in various wood pulps by swelling of both lignified and purified fibers. RITTER (31, 32), by alternate treatment with phosphoric acid and alcohol, has demonstrated balloons, cell-wall lamellae, and a banded outer sheath in elm and similar structures in delignified spruce fibers (30). His photographs of this phenomenon are similar to those shown by both NODDER and HOCK for flax. RITTER stated further than the outer layer of the wood fiber is actually composed of tiny threadlike structures which may be unwound. When this layer has been dissolved, an extreme transverse swelling of the inclosed layers of the wall occurs, and balloon-like structures are formed. He pointed out that such a contrast in the behavior of the two kinds of wall layers strongly suggests a difference in structure.

Similar structures were demonstrated by both ALDABA (1) and NODDER (27) for ramie and by BAILEY and KERR (6) for the tropical species *Olmediella betschleriana* and *Pandanus odoratissimus*.

LÜDTKE (23) also showed balloons in lignified pine-wood tracheids which were indistinguishable from similar balloon structures in cotton fibers, and BIXLER (9) presented photomicrographs of spruce-wood fibers taken from paper pulp, which, when swelled with cuprammonium hydroxide, give ballooning.

The concentric rings of cell-wall layers in swollen cross sections of fibers were shown by BAILEY (5) for pine and larch in a delignified fiber swollen in dilute cuprammonium hydroxide and also for a lignified larch fiber swollen with 75% sulfuric acid. Typical ballooning, parallel layers of the cell wall, and a wrinkled lumen wall were demonstrated by HOCK and SEIFRIZ (19) for purified paper fibers (presumably spruce wood) treated with 85% phosphoric acid. NAKASHIMA (25)

has shown similar ballooning for cotton, Manila hemp, and wood-pulp fibers swelled in cuprammonium hydroxide.

CARPENTER and LEWIS (10), working on fibers from paper pulp, followed the changes in the structure of wood fibers as the result of cooking and bleaching operations. Photomicrographs of the typical wall pattern of "outer sheath" and balloons were presented for aspen, spruce, and jack-pine fibers. The only previous work reported on the cell-wall details of milkweed bast is that of DIPPEL (13), which contains no photographs. He included line drawings which are corroborated by the photomicrographs accompanying the present report.

Material and methods

The milkweed fibers were from the pericycle of unretted stems of the giant milkweed (*A. syriaca*) from which they were separated by boiling in water and in 3% sodium hypochlorite. They were examined both in the raw and in the bleached condition.

The cottons examined contained samples from all three of the main categories of typical commercial growths—Asiatic (*Gossypium herbaceum*), Upland (*G. hirsutum*), and Sea Island (*G. barbadense*)—and included both lint and fuzz fibers.

The milkweed and cotton fibers were studied by swelling with such reagents as concentrated sulfuric acid, phosphoric acid, trimethylbenzyl-ammonium hydroxide, or cuprammonium hydroxide. The most satisfactory was found to be cuprammonium hydroxide of the composition specified by the Committee on Viscosity of Cellulose of the American Chemical Society (2) (30 gm. of copper and 165 gm. of ammonia per liter), diluted to about one-third strength. The fibers were stained, then mounted in water, and the swelling agent drawn under the cover slip by means of filter-paper wicks. If swelling proceeded too rapidly, the action was checked by dilute alcohol or alcohol–acetic acid.

No set schedule of histological procedures can be stipulated, since fibers of different degrees of maturity react differently. The strength of the reagents is determined empirically; during swelling they may be diluted by the addition of ammonia to avoid loss of the specimen through solvation. Care and patience in manipulation of the swelling agent to induce separation of the fiber elements without distortion or rupture are required.

Because of the transparency of the swollen fiber, much of the visible structure is difficult to photograph; and staining, therefore, was used to demonstrate the different structures. The stains used for bringing out the primary and lumen walls as distinct from the layers of cell wall which make up the bulk of the fiber were ruthenium red, methylene blue, congo red, or safranin. In the secondary wall layers staining was of no avail. In many fibers the lumen membrane takes the same stains as does the outer sheath; and the protoplasmic residue, when present, is made conspicuous by its affinity for these dyes. However, no specific information as to chemical composition of the different cell-wall layers is gained from staining.

In addition to raw fibers swelled with cuprammonium hydroxide, fibers were also examined from samples which had been pretreated by oxidation with nitrogen dioxide gas or by purification to α-cellulose by boiling with sodium hydroxide. These chemical treatments served to render the cell-wall elements more readily susceptible to swelling reactions, making it possible to see certain

of the structures more clearly than in the untreated fibers.

Discussion

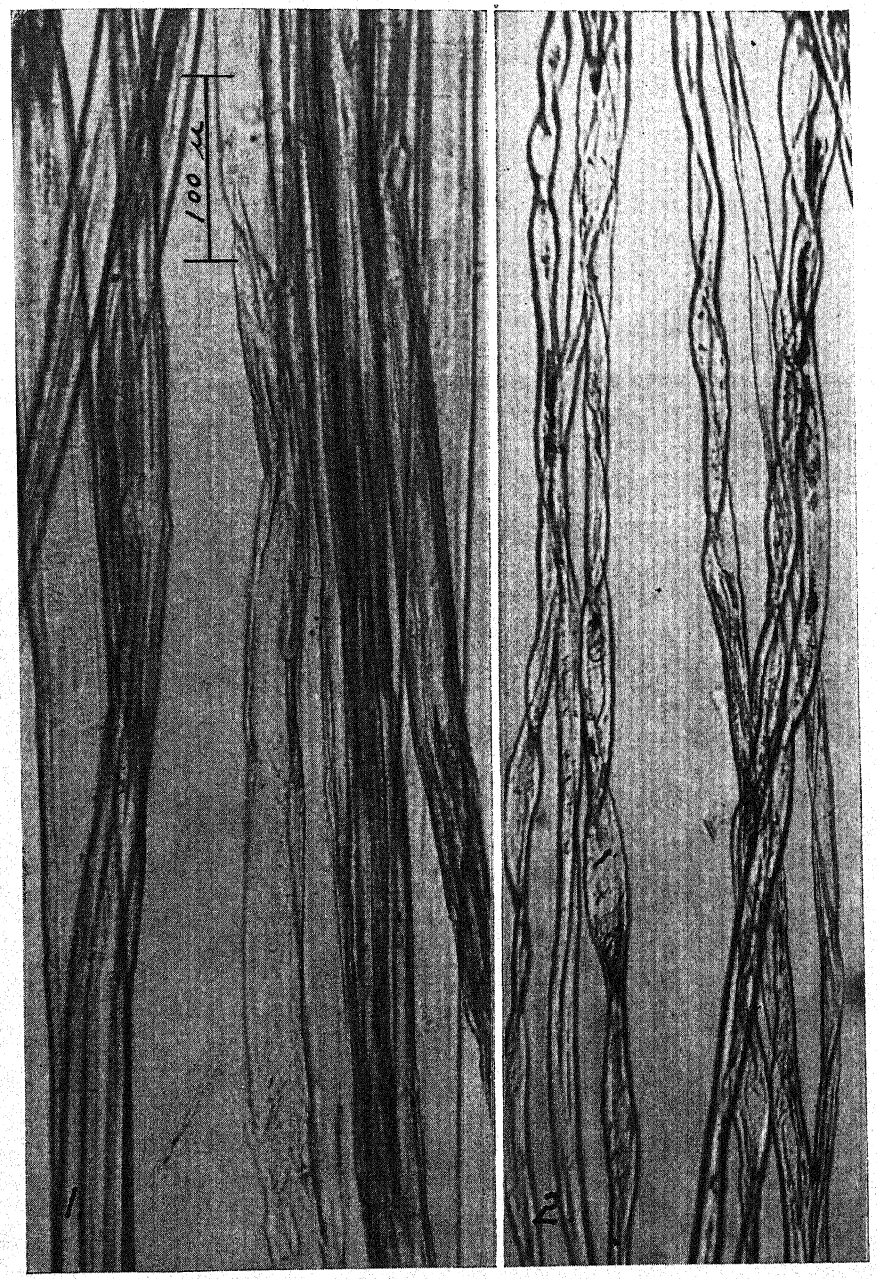
In the individual bast fiber of milkweed the single cell is roughly cigar-shaped with round-pointed ends and, in the desiccated stem, has become collapsed and wrinkled at many points along the fiber length (fig. 1). The fibers range from 15 to 50 mm. in length and from 12 to 30 μ in width, tapering to 1 or 2 μ at the points. The seed hairs of cotton (fig. 2) are much more convoluted and considerably finer in diameter, ranging from 9 to 24 μ in diameter and from 20 to 50 mm. in length, depending on the variety of cotton. There seems to be no outstanding difference in gross structural design between cottons of different varieties or growths or between lint and fuzz fibers.

Ballooning

When swelled in cuprammonium hydroxide, both milkweed and cotton fibers expand rapidly and burst open, the pressure exerted by the cellulose of the swelling wall being great enough to rupture the outer sheath of the fiber. This outermost layer is a thin transparent membrane which, when broken, often rolls up on itself to form tight strings or floats off in large flakes (figs. 3, 4). The rapidly swelling cellulose of the layers of the secondary wall forces itself through the ruptured areas of the primary wall but is prevented from complete swelling by the unbroken portions which form constricting bands. Most frequently the outer sheath breaks at rather regularly spaced intervals, and the consequent swelling of the inner cellulose into "balloons" (fig. 6) accounts for the "beading" or "pearling" figures commonly reported in cotton literature. Similar photographs have been published for flax by Hock (17), for spruce and aspen by Carpenter and Lewis (10), and for Manila hemp by Nakashima (25). In the balloons of milkweed bast (fig. 5) may be seen also the distinct layers of the secondary wall which were inclosed by the outer sheath, and their twisted condition at the constrictions is some evidence of the independent action of each individual layer.

In raw cotton the wall layers are so numerous and so closely packed that they are seldom observed inside the balloons (fig. 6) but may be plainly seen in later stages of the swelling, as in the fuzz fiber of figure 4. Although the number of layers in the secondary wall of the milkweed bast appears to be only five or six, a cotton fiber may have as many as twenty-five (fig. 7), depending on the age in days of growth. Pretreatment of the fibers with gaseous nitrogen dioxide (33), followed by swelling with cuprammonium hydroxide, demonstrated the layering of the secondary wall and a spiraling rupture of the outer sheath (figs. 8, 9). The large number of layers in the cotton fiber and their swelling almost to the point of fusion before they can be discerned as distinct layers are contrasted with the fewer number of readily separable layers in the milkweed fiber. Also seen in these figures are the spiral wrapping in both fibers, into which the outer sheath splits, and the prominent lumen at the center of the cotton fiber brought out by staining with methylene blue.

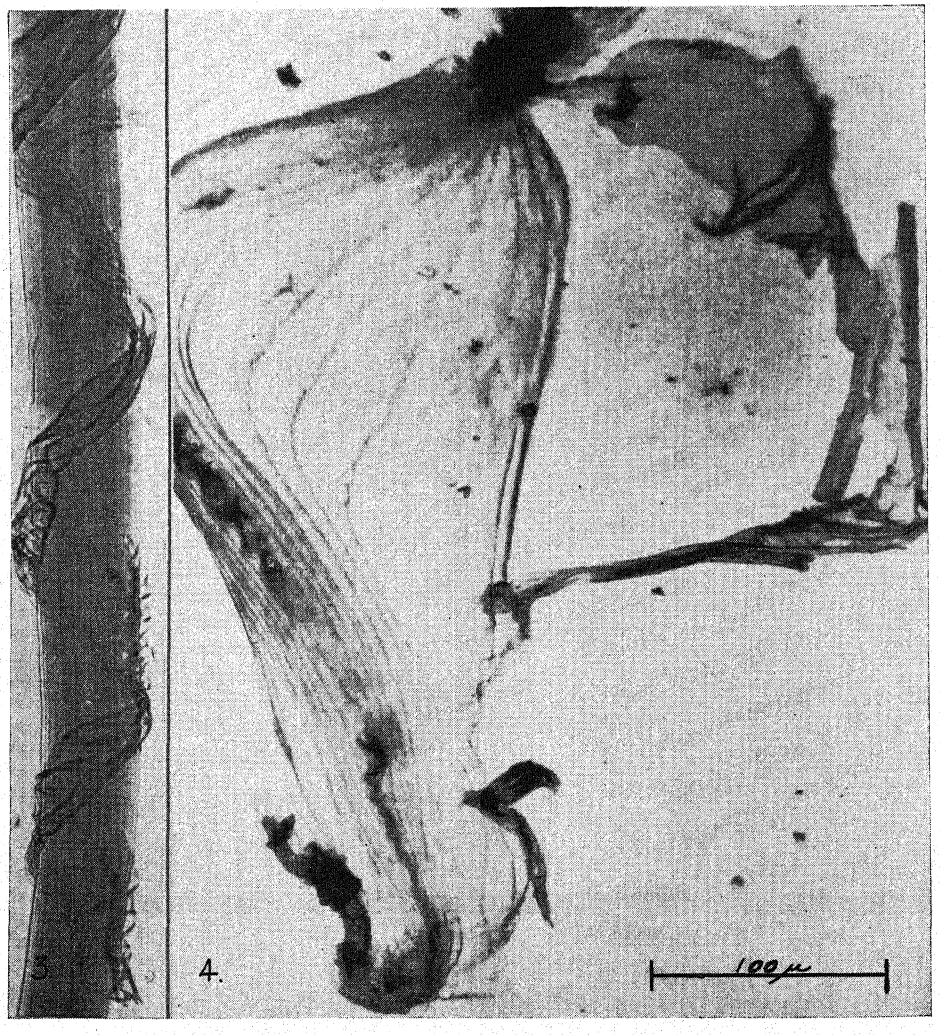
That the outer sheath of the fiber is somewhat elastic is indicated by its rolling-back upon itself when ruptured (figs. 10, 11). A comparative estimate of the extent of swelling of the outer sheath and of the inner cellulose layers shows that "magnification" of the inner walls resulting from swelling in cuprammonium hydroxide solution is in this case approximately eight times. Again the dark-stain-



FIGS. 1–2.—Untreated fibers. Fig. 1, milkweed bast. Fig. 2, cotton lint

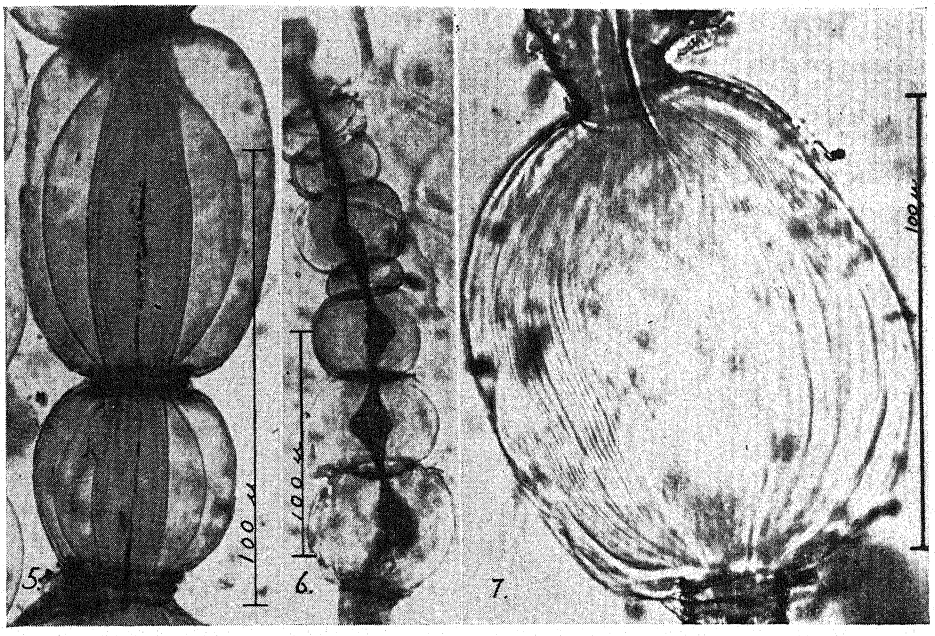
ing residue in the lumen of the cotton fiber (fig. 11) is prominent. It is significant that these photographs are similar to those published previously for cotton (7, 14, 16), spruce (9), elm (31), and an unidentified wood-pulp fiber (19).

Separation of the wall layers is plainly shown in the tips of fibers swollen in cuprammonium hydroxide (figs. 12, 13). The tips of individual layers may be seen telescoped one inside the other like a nest of thimbles. In the cotton fiber may be seen a fragment of the outer sheath tip which formerly inclosed all these layers of the secondary wall; and the large lumen with its heavy residue is even more strikingly prominent. Such photographs as these are difficult to explain by KERR's theory of the "alternating bands of compact and porous cellulose forming a coherent matrix." It is, of course, possible that the porous cellulose which



FIGS. 3–4.—Fibers swollen to show rupture of outer sheath. Fig. 3, milkweed bast. Fig. 4, cotton fuzz

joined these layers (20) has been dissolved by the swelling agent. On the other hand, the photographs appear to support the theory of ALDABA (1), who, in his work on flax and ramie, found the cell wall to be formed by a series of telescoping tubular membranes which gradually become transformed into cell-wall lamellae. Figures 14 and 15 show portions of milkweed and cotton fuzz fibers, respectively, where the wall layers seem to be discrete.



FIGS. 5–7.—Ballooning of swollen fibers caused by constrictions of unbroken outer sheath. Fig. 5, milkweed bast; note twisted and wrinkled condition of inner layers of secondary wall at constrictions. Figs. 6, 7, cotton lint.

LUMEN WALL

The layer lining the lumen often behaves differently from the other wall layers. It resists swelling and frequently stains similarly to the outer sheath of the fiber. The fibers shown in figures 16 and 17 had been pretreated with nitrogen dioxide gas before swelling with cuprammonium hydroxide. The oxidized secondary wall has fused so that no lamellate structure is discernible, but the more resistant lumen wall has not yet swelled enough to unwrinkle the shriveled membrane of which it is composed.

Figure 18 shows swollen cross sections of a group of milkweed bast fibers, remarkably similar to the cross section of an onion. Although fibers of many different ages are represented, some of which were cut near the tip and others at the widest portion of the fiber, it is obvious that the maximum number of layers in any fiber is seven. Figure 19 shows a cotton fiber cross section similarly swelled to show the numerous fine wall layers in the secondary wall. Further photomicrographic evidence of these characteristic lamellae was presented by KERR (20) for cotton, by BAILEY (6) for

larch and pine, and by HOCK (17) for flax.

OUTER SHEATH

Figures 20 and 21 show a single milkweed bast fiber swollen in cuprammonium hydroxide and photographed at two different levels of focus. In figure 20 may be seen the inner wall layers inclosed within the outer sheath or primary wall. Figure 21 shows the top surface of the outer sheath and indicates that it has a definite structure of spiral bands. It is not clear whether the banding is the actual pattern of the outermost sheath or whether it is a pattern induced in the continuous outer membrane by a spiral structure beneath it, which, though separate, swells enough to cause its rupture in a spiral manner. As one follows the course of the spiral, it would appear that it winds in a double ribbon to cover the surface of the fiber.



FIGS. 8–9.—Chemically treated fibers swollen to show spiral winding of outer sheath inclosing lamellae of secondary wall. Fig. 8, milkweed bast. Fig. 9, cotton lint.

Figures 22, 23, and 24 show spiral bandings in the outer sheath of cotton lint fibers which resemble the structure for milkweed bast (figs. 20, 21). Evidence that these bands are not chemically induced artifacts is given by photomicrographs of similar structures in pine fibers by CARPENTER and LEWIS (10), in cotton by DENHAM (12), LAWRIE (22), CLEGG (11), and OSBORNE (28), in flax by NODDER (27), and in spruce by RITTER (32).

Recently MITCHELL (24), in a paper on the nitrated cellulosic constituents of wood, has published photomicrographs of cell-wall "lignin residues" produced in the swelling of nitrated wood fibers with ethyl acetate which show the same spiraling pattern of double bands.

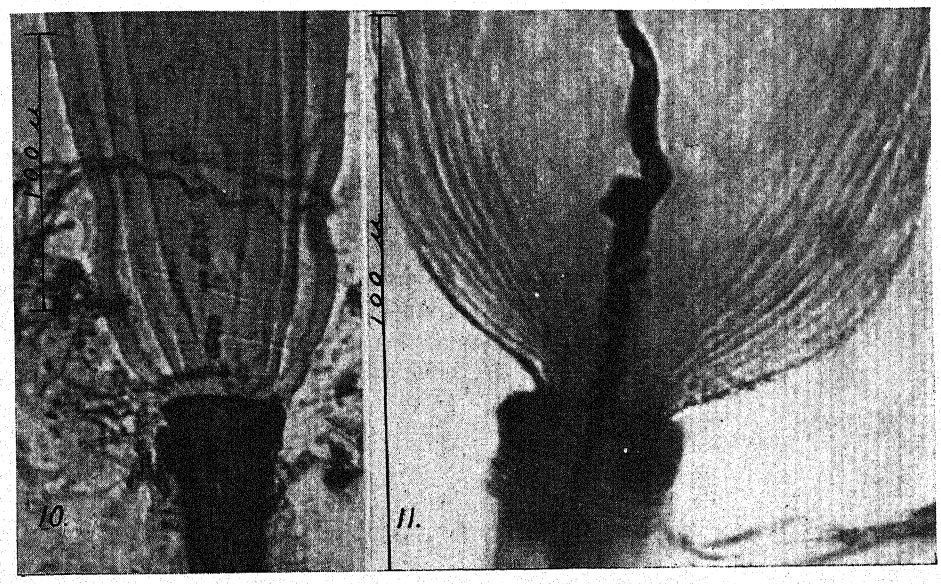
It has not been determined whether the spiral banding apparent in figures 22–24 is a structure of the outer sheath of the fiber or is a pattern in the primary wall caused by the swelling of a spirally wrapped thread just beneath it. It would appear from figure 23, however, that the spiral band is a pattern of the outer sheath itself. This unusual view of a cotton fiber was obtained by oxidation of the fiber in nitrogen dioxide gas for the excessively long treatment of 74 hours, after which the major part of the fiber was readily dissolved in dilute cuprammonium hydroxide, while an outer portion remained more or less intact as a solid shell. Evidently the prolonged treatment so deteriorated the cellulose of the secondary wall that it exerted no swelling pressure to rupture the fiber sheath. On the other hand, in the un-

treated fuzz fiber (fig. 4), swelling pressure of the layers of the secondary wall was so great as to rupture the primary sheath longitudinally rather than in spirals.

In milkweed the spiral banding is more plainly shown in figure 25, in which three of the inner layers of the fiber have escaped through a rupture in the outer sheath to leave the spiraled tip an empty shell.

The chemically treated cotton fiber in figure 26 shows the stained primary sheath pared away to disclose the transparent swollen cellulose of the secondary wall in parallel lamellae on either side of the lumen.

Although in dried cotton it has not been possible to photograph the spiral direction of the finer structural elements of which each lamella is composed (cf. ANDERSON and KERR [4]), figure 27



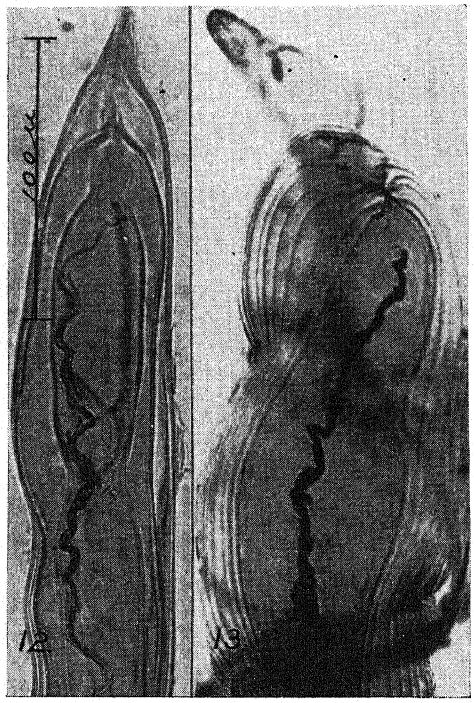
FIGS. 10–11.—Fibers swollen to show lamellae issuing from inclosing primary sheath. Fig. 10, milkweed bast. Fig. 11, cotton fuzz fiber. Note rolled sheath at base of swollen portion.

Although it has not been possible to photograph for cotton, as was done for milkweed (figs. 21, 25), the lamellae inclosed within the spiraling outer sheath, it is evident from figures 22–24 that a similar structural design exists for both types of fibers. In figure 24 the unswelled portion of the cotton fiber protected by the primary sheath is stained with methylene blue, and the broken sheath can be seen separating into a spiral wrapping.

shows the fine spiral threads in a wall layer of a milkweed bast fiber lying at an angle to the fiber axis. KERR (21) has recently photographed this same phenomenon in mature but undried cotton fibers from a green boll. It must be assumed that refractive indices in dried cotton fibers are so changed that photographic evidence is extremely difficult to obtain or that swelling obscures this fine structure by fusing the fibrils into a solid mass.

Kapok and milkweed floss, though

technically seed hairs and morphologically parallel to cotton, did not respond to swelling solutions in the typical manner described in this paper. This was undoubtedly because they lack sufficient layers of cellulose to produce swelling pressures great enough to rupture the outer wall. The presence of waterproofing materials deposited in the outer sheath



FIGS. 12–13.—Closed tapered tips of swollen fibers. Fig. 12, milkweed bast. Fig. 13, cotton fuzz fiber. Note cell-wall layers emerging from ruptured primary sheath.

was also a contributing factor, though milkweed floss fibers from which the wax had been extracted failed to swell in cuprammonium hydroxide.

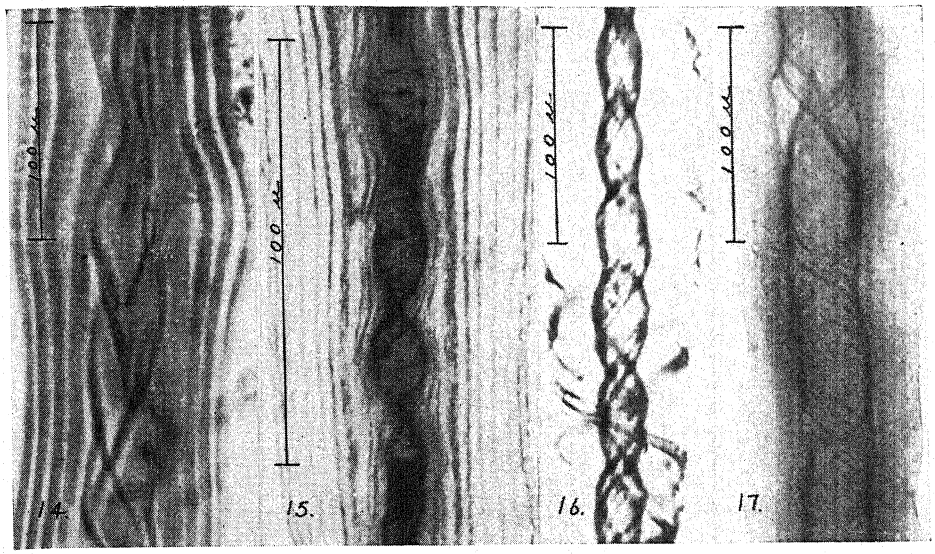
Summary

1. The similarity between two widely different types of fibers such as milkweed bast and seed hair of cotton illustrates a basic principle of nature in the structural design of cell walls of the fibrous type.

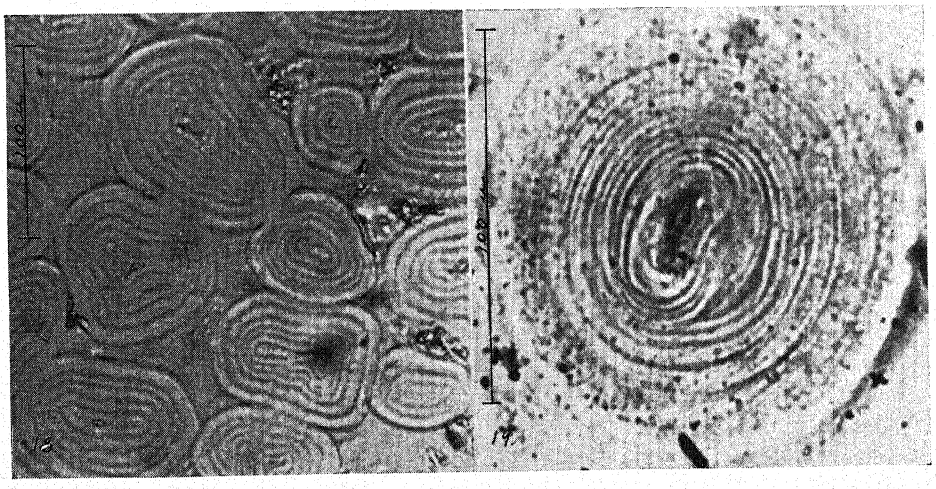
2. The photomicrographs here presented support in general the findings of other workers in the field of microscopical cell-wall structure. It is established that the cell wall consists of (*a*) an outer sheath, which is hardly capable of any appreciable swelling; (*b*) a many-layered cellulose wall which may be swelled ten to twenty times its original size before solvation; and (*c*) an inner canal inclosed by a lumen wall which in its reactions is more like the outer membrane of the fiber than it is like the secondary-wall layers. The fiber sheath appears to have a banded structure when not ruptured by swelling of the cellulose beneath it. In addition to confirming previous observations, these photomicrographs indicate that the cell-wall layers of the secondary wall in both seed hairs of cotton and milkweed bast fibers are discrete membranes, readily separable one from the other by swelling reagents. The photomicrographs further serve to co-ordinate information acquired by various investigators working on individual fibers from diverse natural sources.

3. Although no spiral structure was observed in any layer of the secondary wall of cotton, it is probable that the spiraling fibrils are so fine as to be fused by the swelling action or are rendered indistinguishable by their refractive index. Spiral fibrils were photographed in a layer of the secondary cell wall in a nitrogen dioxide–treated milkweed fiber after swelling in cuprammonium hydroxide.

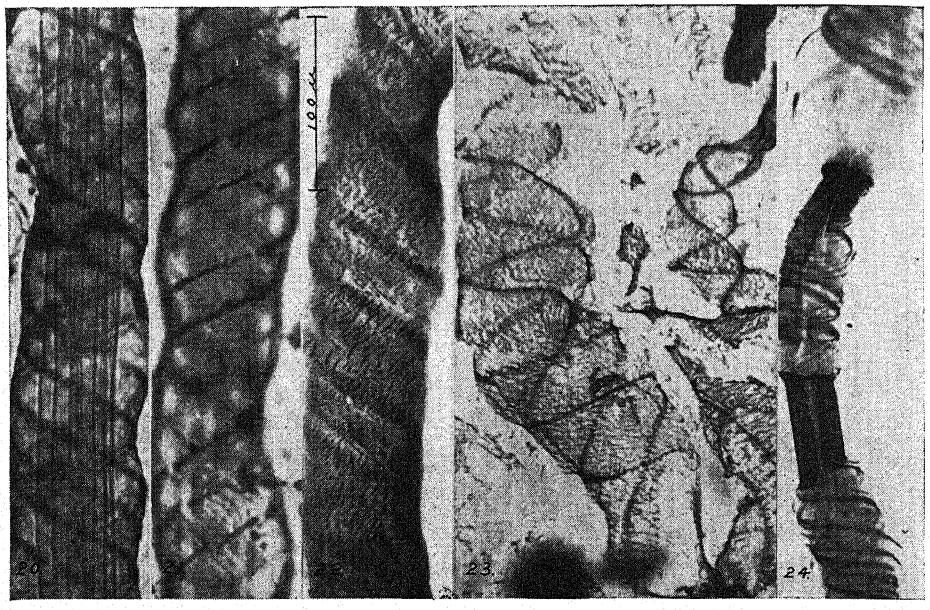
The author wishes to express her appreciation for the interest and advice of Mr. T. L. W. BAILEY, JR., in the details of the microscopical work; and for the



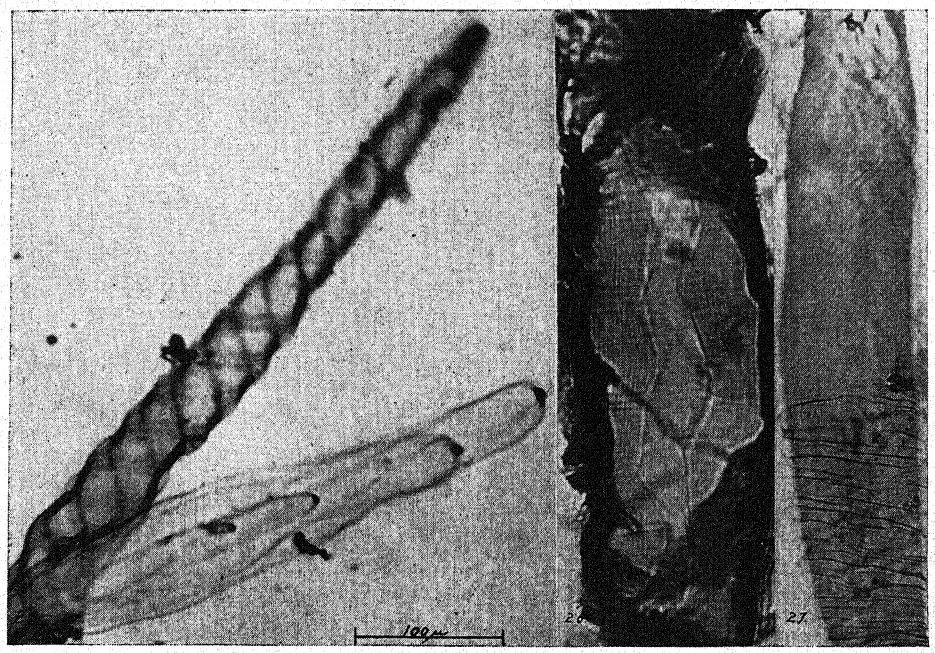
FIGS. 14–17.—Fibers swollen to show layers of secondary wall and convoluted lumen. Fig. 14, chemically treated milkweed bast. Fig. 15, cotton fuzz fiber. Fig. 16, chemically treated milkweed bast. Fig. 17, chemically treated cotton lint.



FIGS. 18–19.—Swollen cross sections of fibers showing layers of secondary wall. Fig. 18, milkweed bast fibers. Fig. 19, cotton lint.



FIGS. 20–24.—Fibers swollen to show banding in outer sheath. Figs. 20, 21, milkweed bast with focus at center and top planes, respectively. Figs. 22, 23, 24, chemically treated cotton lint. Fig. 22, focus at top plane. Fig. 23, residue after solvation of fiber. Fig. 24, uneven rupturing of outer sheath into spirals.



FIGS. 25–27.—Chemically treated fibers swollen to show inner structure. Fig. 25, milkweed bast. Note inner layers released from ruptured spiral sheath. Fig. 26, cotton lint. Note lamellae disclosed by peeling back of ruptured sheath. Fig. 27, milkweed bast. Note fibrillar structure of cell-wall layer beneath broken spiral of outer sheath.

assistance of Miss INES V. DE GRUY in photomicrography and in the preparation of the manuscript. Acknowledgment is also due Dr. R. E. REEVES and his co-workers for the nitrogen dioxide treatment of the fibers.

SOUTHERN REGIONAL RESEARCH LABORATORY
NEW ORLEANS, LOUISIANA

LITERATURE CITED

1. ALDABA, V. C. The structure and development of the cell wall in plants. I. Bast fibers of *Boehmeria* and *Linum*. Amer. Jour. Bot. 14: 16–24. 1927.
2. AMERICAN CHEMICAL SOCIETY COMMITTEE ON THE VISCOSITY OF CELLULOSE. A standard method for determining the viscosity of cellulose in cuprammonium hydroxide. Ind. Eng. Chem. (Anal. Ed.) 1:49–51. 1929.
3. ANDERSON, D. B. A microchemical study of the structure and development of flax fibers. Amer. Jour. Bot. 14:187–210. 1927.
4. ANDERSON, D. B., and KERR, THOMAS. Growth and structure of cotton fiber. Ind. Eng. Chem. 30:48–54. 1938.
5. BAILEY, I. W. Cell wall structure of higher plants. Ind. Eng. Chem. 30:40–47. 1938.
6. BAILEY, I. W., and KERR, THOMAS. The visible structure of the secondary wall and its significance in physical and chemical investigations of tracheary cells and fibers. Jour. Arnold Arboretum 16:273–300. 1935.
7. BALLS, W. L. The existence of daily growth-rings in the cell wall of cotton hairs. Proc. Roy. Soc. (London), Ser. B, 90:542–555. 1919.
8. BALLS, W. L., and HANCOCK, H. A. Cutting sections of cotton hairs. Nature 107:361. 1921.
9. BIXLER, A. L. M. Effect of digestion on wood structure. Paper Trade Jour. 107(15):29–40. 1938.
10. CARPENTER, C. H., and LEWIS, H. F. Changes in the structure of wood fibers during cooking and bleaching. Paper Trade Jour. 99(3):37–42. 1934.
11. CLEGG, G. G. The examination of damaged cotton by the congo red test: further developments and applications. Jour. Text. Inst. 31:T49–68. 1940.
12. DENHAM, H. J. The structure of the cotton hair and its botanical aspects. II. The morphology of the wall. Jour. Text. Inst. 14:T86–113. 1923.
13. DIPPEL, LEOPOLD. Das Mikroskop und seine Anwendungen. Anwendung des Mikroskopes auf die Histologie der Gewächse, Vol. 2, 2d ed. Braunschweig. 1898.
14. FARR, WANDA K. Behavior of the cell membrane of the cotton fiber in cuprammonium hydroxide solution. Contrib. Boyce Thompson Inst. 10:71–112. 1938.
15. HANSON, H. B. Structure, properties, and preparation of certain bast fibers. Iowa State College Jour. Sci. 20:365–383. 1946.
16. HOCK, C. W. Microscopic structure of the cell wall. Pp. 11–21 *in* The structure of protoplasm. A monograph of the American Society of Plant Physiologists. Iowa State College Press, Ames, Iowa. 1942.
17. ———. Microscopic structure of flax and related bast fibers. Jour. Res. Nat. Bur. Stand. 29:41–50. 1942.
18. HOCK, C. W.; RAMSAY, R. C.; and HARRIS, M. Microscopic structure of the cotton fiber. Jour. Res. Nat. Bur. Stand. 26:93–104. 1941.
19. HOCK, C. W., and SEIFRIZ, W. Microdissection of paper pulp fibers. Paper Trade Jour. 110(5): 31–32. 1940.
20. KERR, THOMAS. The structure of the growth rings in the secondary wall of the cotton hair. Protoplasma 27:229–241. 1937.
21. ———. The outer wall of the cotton fiber and its influence on fiber properties. Text. Res. Jour. 15:249–254. 1946.
22. LAWRIE, L. G. The microscopical investigation of textile fibers and fabrics. Jour. Text. Inst. 20:P99–105. 1929.
23. LÜDTKE, M. Bau der Pflanzenzellwand und dadurch bedingte Erscheinungen. Zellst. u. Pap. 15:273–276, 320–323. 1935.
24. MITCHELL, R. L. Chain length measurements on nitrated cellulosic constituents of wood. Ind. Eng. Chem. 38:843–850. 1946.
25. NAKASHIMA, T. Viscose. XXVI. Swelling of cellulose in sodium hydroxide solutions. Cellulose Ind. (Japan) 5:199–202. 1929.
26. NELSON, E. G., and DEXTER, S. T. Fiber from the stems of common and swamp milkweeds. Michigan Quart. Bull. 28:20–26. 1945.
27. NODDER, C. R. A study of flax and kindred fibres. I. Some observations on the behaviour and structure of textile fibres, with an account of a convenient method of distinguishing flax from hemp. Jour. Text. Inst. 13:161–171. 1922.
28. OSBORNE, G. G. Micro-analysis of textile fibres. Part III. Observations on the structure of cotton. Text. Res. 5:307–325. 1935.
29. OTT, EMIL (ed.). Cellulose and cellulose derivatives. Interscience Publishers, Inc., New York. 1943.
30. RITTER, G. J. Wood fibers. Jour. Forestry 28:533–541. 1930.

31. RITTER, G. J. Structure of the cell wall of wood fibers. Paper Ind. 16:178–183. 1934.
32. ———. The morphology of cellulose fibers as related to the manufacture of paper. Paper Trade Jour. 101(18): 92–100. 1935.
33. ROLLINS, M. L. Applications of nitrogen dioxide treatment to the microscopy of fiber cell wall structure. Text. Res. Jour. 15:65–77. 1945.
34. VAN ITERSON, G., JR. The development of plant cell-wall. (Translated by MARIE P. LÖHNIS and ELOISE GERRY.) Chem. Weekblad 24: 166–187. 1927.
35. ———. Biologische inleiding tot het cellulose—symposium ["Biological introduction to the cellulose symposium"]. Chem. Weekblad 30: 2–19. 1933.

PERSISTENCE OF 2,4-DICHLOROPHENOXYACETIC ACID IN SOIL IN RELATION TO CONTENT OF WATER, ORGANIC MATTER, AND LIME[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 583

OLIVE H. KRIES

Introduction

The increasing use of 2,4-dichlorophenoxyacetic acid (2,4-D) in spray applications to plants for various purposes and the possibilities in expanding its use to include direct treatment of soil necessitate the investigation of its possible effects upon and persistence in soils of diverse types in relation to various management practices. Experiments reported upon to date (**1, 4, 5, 6, 7, 8, 11, 12**) indicate that the length of persistence in soil of the toxic effects to higher plants varies with soil type and management as well as with the amount applied, the method of application, and the kind of plant.

The present experiment was devised to secure additional data on the persistence of activity of 2,4-D in one soil in relation to its content of water, organic matter, and lime. It was carried on in the University of Chicago greenhouses from March 27 to August 31, 1946.

Material and methods

Heavy black loam from northern Illinois was mixed with coarse sand in the proportion of 3:1 by volume. Approximately 1440 pounds of the mixture were divided into six lots, two of which were allowed to become air-dry; the other four lots were kept moist. Prior to drying, partially decomposed leaf mold was added to one of the lots to be air-dried and to two of the moist lots in the ratio of 1:3 by volume. Lime ($CaCO_3$), at the rate of 1 lb. to 500 lb. of soil, was mixed with two of the four moist lots on March 27. The six lots thus included: dry soil; dry soil + leaf mold; moist soil; moist soil + leaf mold; moist soil + lime; and moist soil + lime + leaf mold. These terms will be used in referring to these lots hereafter.

After preparation all six lots were stored in large boxes in a room adjacent to the greenhouse. The reactions of the various combinations ranged from pH 7.8 to 8.2; moisture equivalents, determined by the centrifuge method, from 19.2% to 21.5%. Water content in the moist lots ranged from 21.5% to 24.3% during the first week of storage.

Meanwhile, 36.0-, 3.6-, and 0.36-gm. quantities of commercial grade 2,4-D were each dissolved in 100 ml. of ethyl alcohol. The three solutions were each then thoroughly mixed by hand with

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1-kg. amounts of white quartz sand which was spread out to allow evaporation of the alcohol.

The six different lots of stored soil were each then divided into four aliquots. To three aliquots of each lot, the dry sand containing 2,4-D was added on April 7 (11 days after the lime had been added) so as uniformly to incorporate 2,4-D into the soil at the rate of 2, 20, and 200 p.p.m., respectively, based on the dry weight of the soil. The fourth aliquot of each lot served as an untreated control. The amount of sand so added was negligible in its effect upon textural and structural relations of the soil.

The twenty-four lots of soil which resulted from the possible combinations of the various treatments were placed in storage in a room not subject to great temperature fluctuations; temperatures 2-4 inches below the surface in the stored soil averaged about 74° F. with a low of 58° on April 26 and a high of 84° on July 8.

Water was added to the moist lots occasionally to offset evaporational losses but not in such quantity as to result in leaching.

On April 14 and 21, May 5, July 17, and August 15, soil from each lot was placed in 5-inch, white, glazed pots provided with glass-wool filters over the drainage hole. Torpedo sand filled the lower one-fourth of each pot. On April 14 and 21 ten pots were so filled from each of the twenty-four stored lots, and on the other dates five pots were filled from each. Thus, soil was taken from each storage lot into the greenhouse 1 week, 2 weeks, 4 weeks, 14½ weeks, and approximately 18 weeks after 2,4-D had been mixed with it.

On each removal date the filled pots were placed on a greenhouse bench, and each pot was uniformly planted with five Red Kidney beans and ten seeds each of barley, African marigold, and white mustard. These species are sensitive in different degree to 2,4-D. The rate and percentage of emergence of the seedlings were used as primary criteria of the degree of toxicity of 2,4-D and of its persistence in the different stored lots on the various planting dates.

Half the number of pots in each treatment in the April 14 planting were replanted on May 12. In this process the soil was allowed to dry to the point where the old plants could be pulled free with little loss of soil. The other pots in the April 14 planting were replanted on June 2. A third planting was made on July 15 on the soil initially planted on April 14 and replanted on May 12.

Similarly, half the number of pots of the April 21 sowing were replanted on May 19. In the cases of two pots of each of the twenty-four treatments, the top and bottom halves of the soil mass were removed to clean, separate pots to test whether residual toxicity differed in the top and bottom layers of the soil.

On August 16 the pots initially planted on July 17 were replanted.

Ten days after each planting all but one of the bean seedlings in the control pots were cut off below the cotyledons so that the smaller seedlings would not be excessively shaded. This procedure was not necessary in the first plantings in the pots containing 2,4-D because of its inhibiting effect on beans.

Counts of germination were made 14 days after each planting. Germination was considered, following PORTER (9), as the emergence and development from the embryo of those essential structures which, for the kind of plant in question, were indicative of the ability to produce a normal plant under favorable conditions. All such data were calculated in percentages and graphed (figs. 1–4). Further development of the plants was fol-

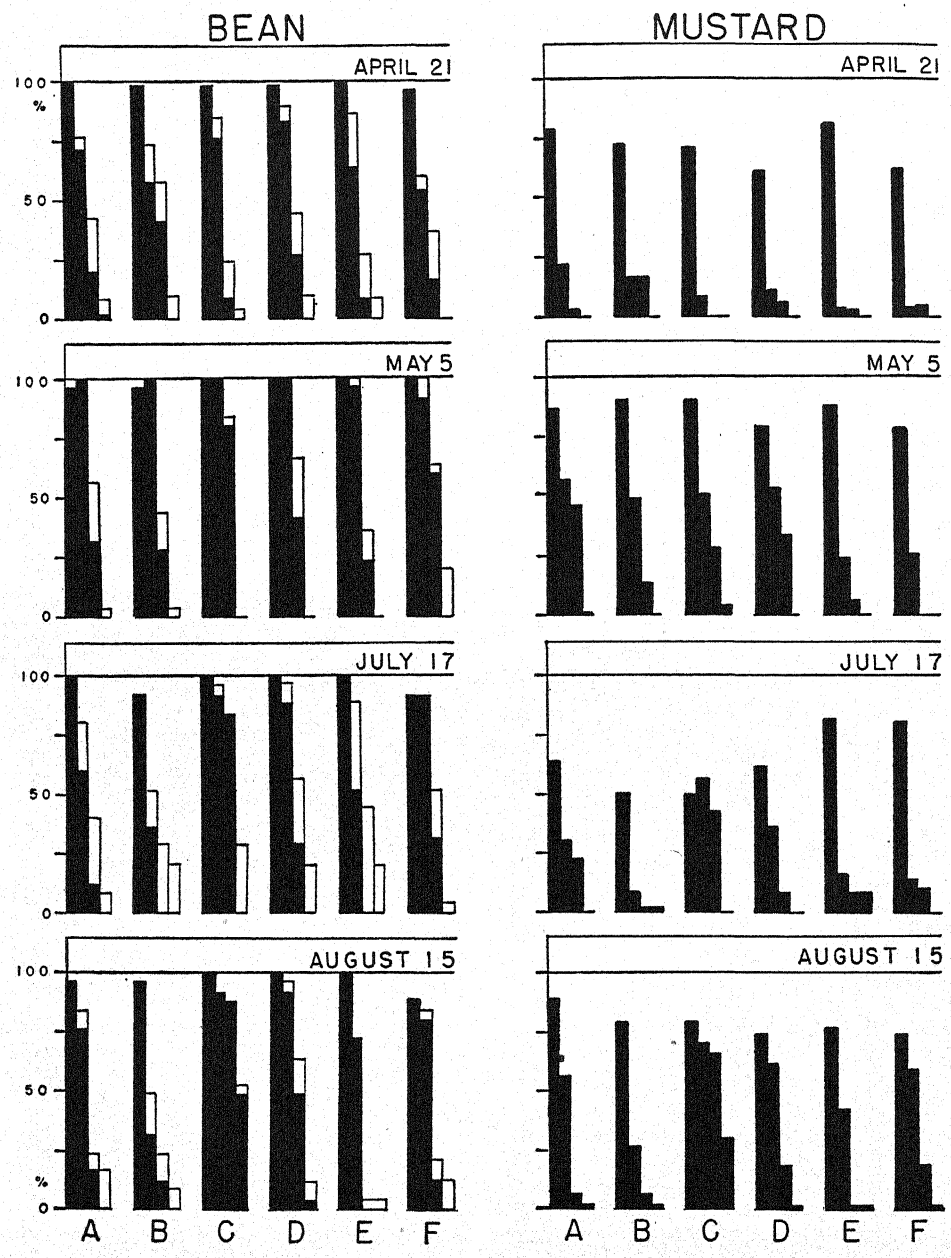


FIG. 1.—Percentages of emergence of test plants 14 days after planting on indicated dates from soil which had been stored since April 7. Conditions of storage were: *A*, dry soil; *B*, dry soil + leaf mold; *C*, moist soil; *D*, moist soil + leaf mold; *E*, moist soil + lime; *F*, moist soil + lime + leaf mold. Each group of four bars, from left to right, respectively, represents emergence from soil in which 2,4-D had been incorporated on April 7 at rate of 0, 2, 20, and 200 p.p.m.

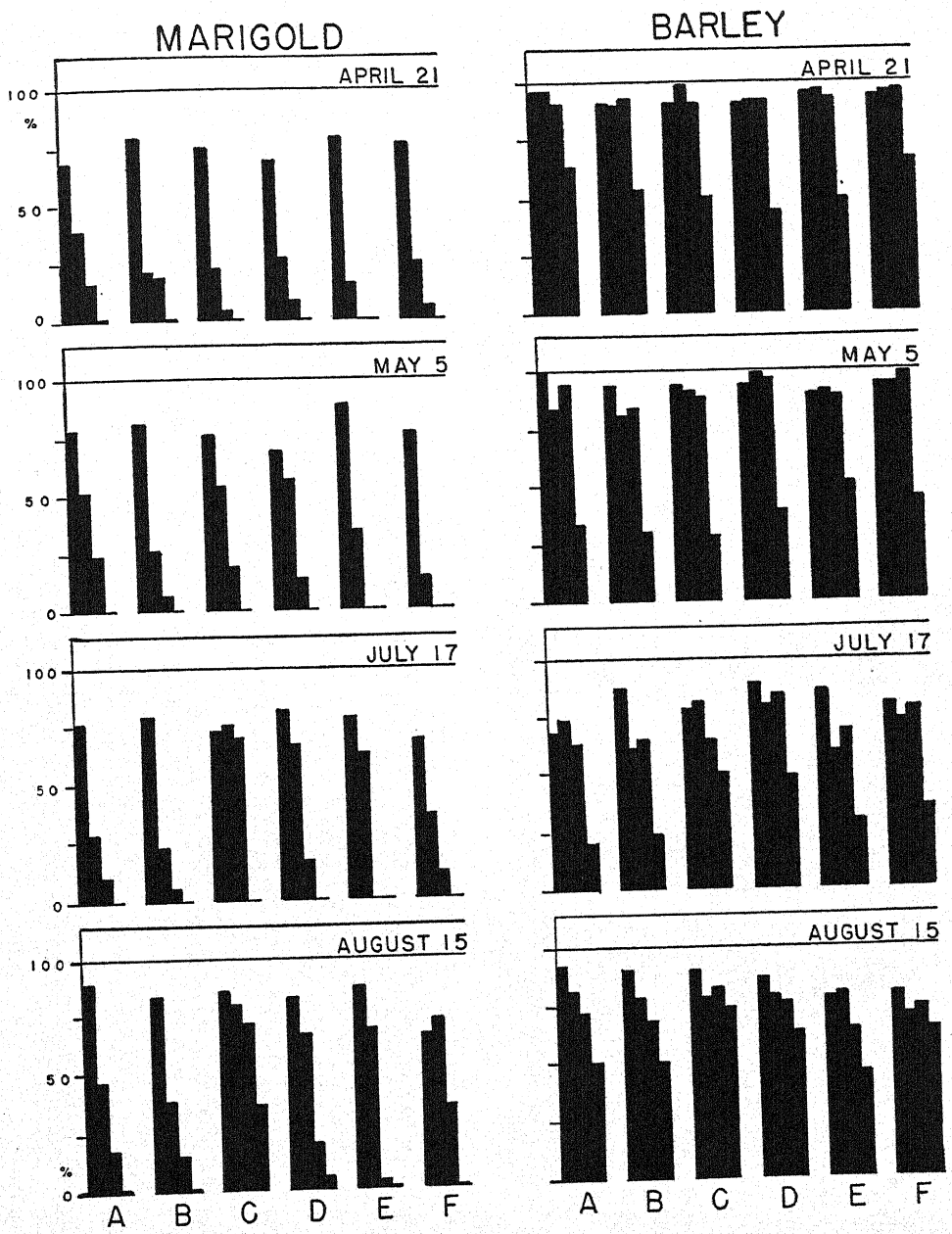


FIG. 2.—As in fig. 1 for other test species

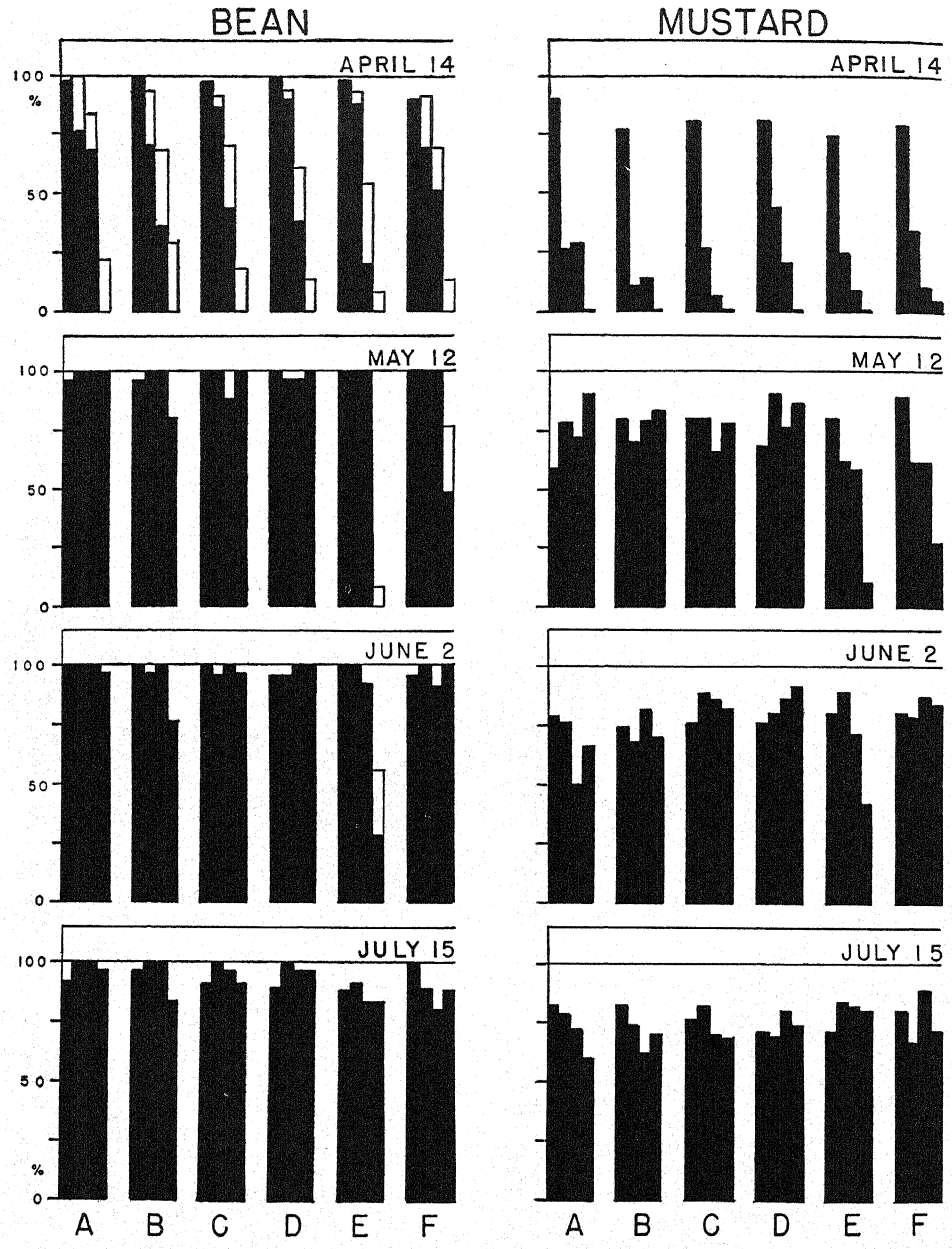


FIG. 3.—Percentages of emergence of test plants 14 days after planting on indicated dates. Each group of four bars and categories *A*, *B*, *C*, *D*, *E*, and *F* as in fig. 1. Planting on April 14 made after 1 week of soil storage. Data for other dates are of replantings on same soil following 4, 7, and 13½ weeks of cropping. Note effect of lime (*E*) on retention of toxicity and of leaf mold (*F*) in mitigating its effect.

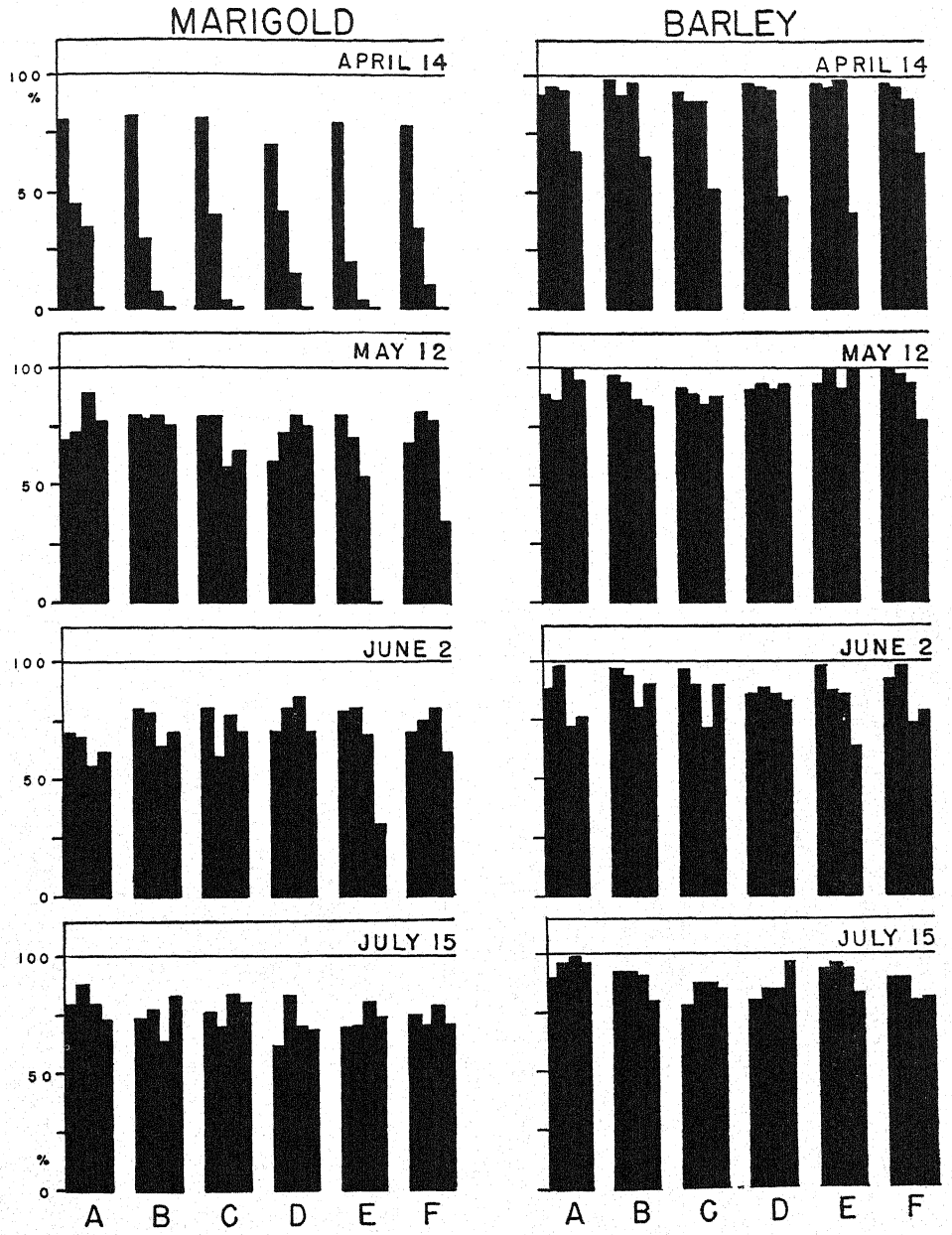


FIG. 4.—As in fig. 3 for other test species

lowed for varying lengths of time after each planting date.

The pots were watered sparingly but frequently so as to avoid leaching of 2,4-D in drainage water. Thus, the eventual loss of toxicity either from the stored soil or from the soil after its removal to the greenhouse and planting was due in part, at least, to factors other than leaching.

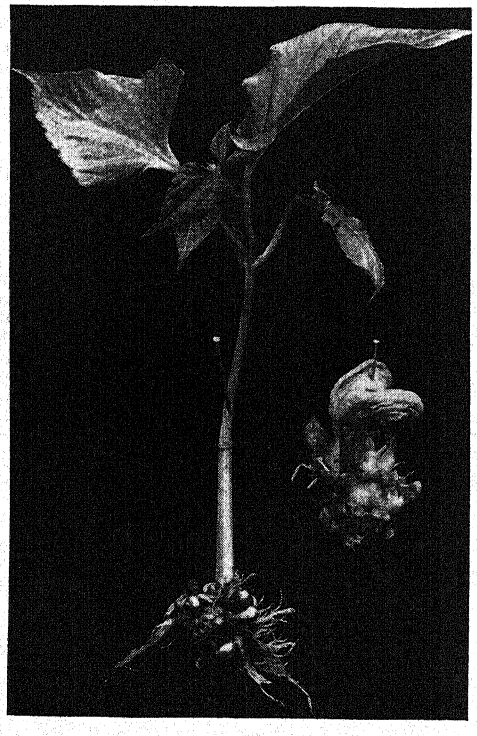


FIG. 5.—Beans 26 days after planting on May 5 in moist soil + lime. *Left*, from 2-p.p.m. (2,4-D) pot; *right*, from 20-p.p.m. pot.

Air temperature in the greenhouse from April 21 until the last week in May varied from 90° to 98° F. at midday to a night minimum of about 74°. During the last week in May and practically all of June, daily maximum readings stood at 98° with one exception. During the first part of the third week of June, temperatures remained close to 80° day and night. A low of 65° occurred twice during the month. Through July 15, noon temperatures reached 100° on an average with a low on one night of 76°. In August, daily maximum temperatures were somewhat lower, with an average of about 95° and one low of 74°.

Naturally, the plants required large amounts of water to offset losses by transpiration during these continued high temperatures. Frequent but light watering was maintained. Soil temperatures in the pots at a depth of 4 inches ranged from 59° to 98° F. during the season.

Results

SOME EFFECTS OF 2,4-D ON SEEDLING DEVELOPMENT

As judged by seedling emergence in this experiment, mustard and marigold seeds were more intolerant of 2,4-D than Red Kidney bean and barley, and marigold seemed to be more sensitive than mustard. The sensitivity of marigold to 2,4-D has been previously reported (2, 3).

Development of bean seedlings deviated from normal to such an extent in all the initial plantings on soils originally containing 20 and 200 p.p.m. of 2,4-D that it was necessary to designate the seriously injured specimens in making the counts. Consequently, the clear spaces in the bar graphs (figs. 1, 3) indicate the percentages of seedlings showing markedly abnormal development, the solid bars those with more nearly normal development. The seriously injured seedlings were often upside down and much contorted (fig. 5). Frequently, they possessed four flanges of tissue at the bases of the hypocotyls. These abnormalities have been described by others (1, 4).

The capacity of substituted phenoxyacetic acids to induce modifications in size, shape, pattern, and venation of leaves was first reported in 1942 (13). In the present experiment the heart-shaped leaves of beans strongly affected by 2,4-D often failed to expand properly and were yellowish-green in color with the veins more or less outlined with small black dots. In general, they appeared stiff and dry. Some of the trifoliate leaves in any later plantings although the irregularities appeared in some of the 2,4-D treatments in all the successive initial plantings on stored soil.

Mustard germinated first in each planting, and those mustard seedlings which emerged from soil treated with 2,4-D exhibited no characteristic abnormalities.

Marigold seedlings developed swollen regions at the bases of the petioles in

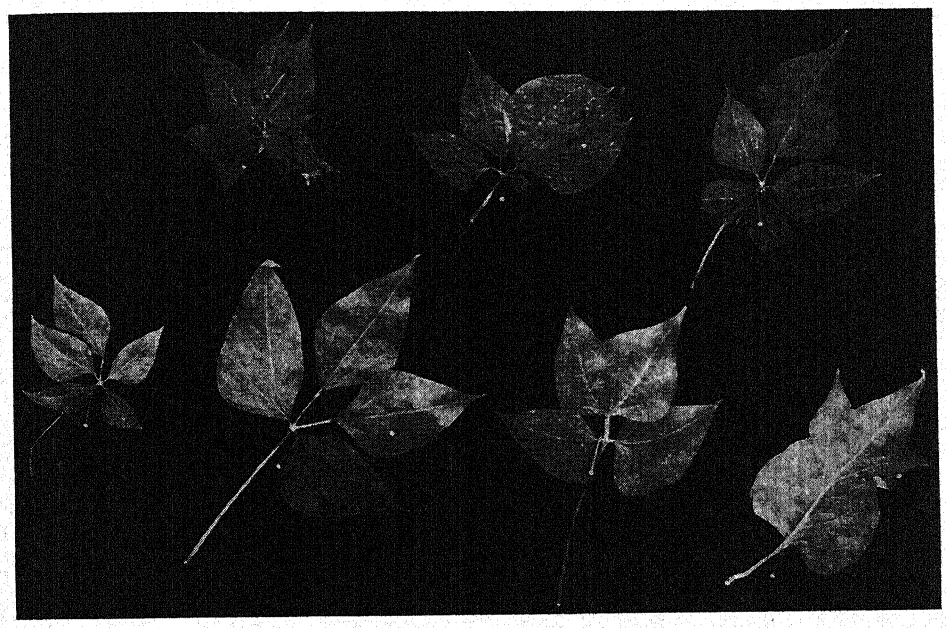


FIG. 6.—First "trifoliate" leaves of beans from various 20-p.p.m. (2,4-D) pots in April 14 planting

of bean displayed telemorphic effects (2, 3) from 2,4-D in the production of excessive numbers of leaflets as well as in the nondivergence of leaflets. Some leaves had one leaflet only, while others had as many as six or eight divisions (figs. 5, 6). No specimens were found with more than ten leaflets per leaf. Judging from counts made on these anomalous leaves of bean, more plants in the first sowing (April 14) in the soil treated with 2 and 20 p.p.m. of 2,4-D possessed this type of malformation than some plants in the initial plantings in soil originally containing 20 p.p.m. of 2,4-D. Occasionally, the leaves seemed narrower than those in the controls. If germination was possible, however, there were no striking morphogenic effects from 2,4-D on either marigold or mustard.

In barley the coleoptiles remained very pale, almost white, for several days in the initial plantings in soil originally containing 200 p.p.m. of 2,4-D. Later, the green plumules emerged weakly and elongated

a few inches. A brown spotting superficially resembling a rust developed on the leaves of plants in the 2- and 20-p.p.m. soils in most of the initial sowings in stored soil. Frequently it was followed by a chlorosis of the entire leaf. A burning of the leaf tip was also rather common in these pots.

Germination in control pots

Some deviations in the percentages of germination in the controls were apparent in relation to season of planting, but the six major soil categories had little effect on the percentages of emergence in control pots on any one planting date. Throughout the experiment Red Kidney bean was high in percentage of germination in all controls. The percentage of germination for mustard was noticeably reduced in the July 17 planting, while the percentage for barley was relatively low in both the July 17 and the August 15 plantings. On the whole, of the four species tested, marigold was lowest in percentages of germination in controls for all ten planting dates. All statements concerning the effects of 2,4-D upon emergence are based on comparisons with the control pots in the same category of soil.

Inhibitory effects of 2,4-D in stored soil

The persistence of the toxic effect of 2,4-D in stored soil is shown in table 1, in figures 1 and 2, and in the data for April 14 in figures 3 and 4, based chiefly on percentages of emergence of test species in the five different plantings on stored soil. The data in table 1 are weighted somewhat in accord with the later development of the seedlings.

Results from soil stored 1 week (figs. 3–4 for April 14) and from soil stored 2 weeks (figs. 1–2 for April 21) were practically identical. Toxicity to bean, marigold, and mustard was apparent at all three levels of concentration of 2,4-D in all six categories of soil treatment. For barley there were no inhibitory effects in the soil originally containing 2 and 20 p.p.m. of 2,4-D in any of the six combinations.

Results from the planting on May 5 (figs. 1–2), after 4 weeks of soil storage, were much the same as in the previous plantings, except that Red Kidney bean showed little or no injury in the 2-p.p.m. soil, and barley appeared to be stimulated in the 20-p.p.m. dry-soil treatment.

In the July 17 planting on soil stored for 14½ weeks, there were no pronounced inhibitory effects on any of the four species in the moist-soil lots at the 2- and 20-p.p.m. levels. All the seeds in the moist-soil lot at 200 p.p.m. were affected, however. The other five major soil categories showed toxicity essentially the same as in the three earlier initial plantings on stored soil.

In the last of the initial sowings on August 15, on soil stored 130 days, pronounced inhibitory effects continued with some exceptions: the lots of moist soil were least toxic of all, and moist soil+leaf mold, with or without added lime, was less toxic than the other three soil combinations.

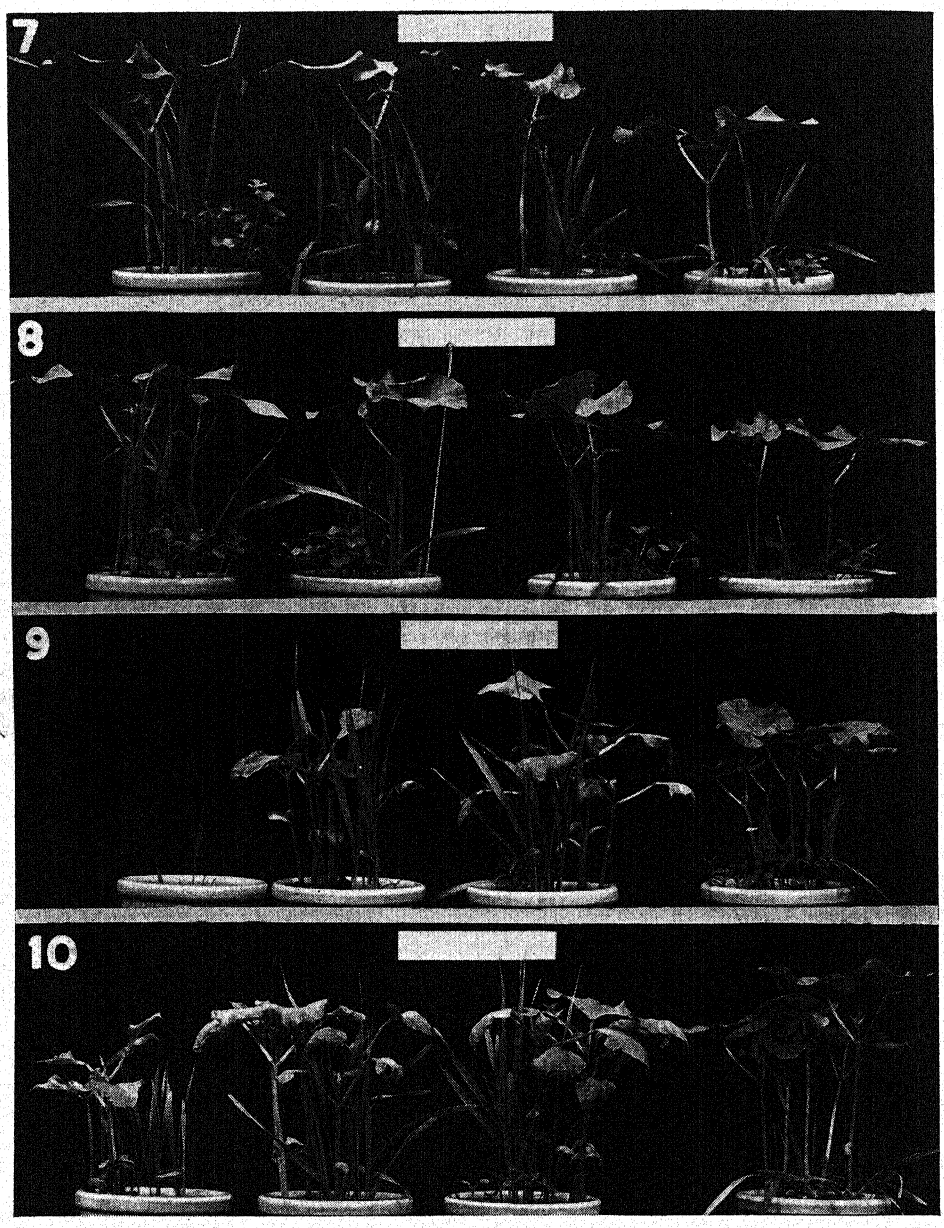
Thus, in general, there was very slight difference among soil lots stored for 1, 2, or 4 weeks in the persistence and degree of toxicity of equivalent amounts of 2,4-D added at the beginning of storage. The persistence was little affected by moisture content of soil, by the status of its lime content, or by the addition of leaf mold. After 14½ weeks of storage, however, there had been a pronounced loss of toxicity in the moist-soil lots which contrasted strongly with the five other categories. There was a gradual

TABLE 1

PRESENCE OR ABSENCE OF TOXIC OR STIMULATING EFFECTS WHICH RESULTED FROM MIXING 2,4-D IN SOIL ON APRIL 7 AS SHOWN BY SEEDLING EMERGENCE AND LATER DEVELOPMENT OF FOUR TEST SPECIES*

Initial soil	2,4-D added (p.p.m.)	Soil in storage April 7–14; then in greenhouse				Soil in storage April 7–21; then in greenhouse		Soil in storage April 7–May 5; then in greenhouse	Soil in storage April 7–July 17; then in greenhouse		Soil in storage April 7–August 15; then in greenhouse
		Planted April 14	Replanted May 12	Replanted June 2	Replanted July 15	Planted April 21	Replanted May 19	Planted May 5	Planted July 17	Replanted August 16	Planted August 15
Dry soil	2	+++−	−−−−	−−− S	S−− S	+++−	−−−−	−++−	+++−	−−−−	−++−
	20	+++−	− S S S	−−−−	S−− S	+++−	− S−−	+++ S	+++−	+−−−	+++−
	200	++++	− S− S	−−−−	−−− S	++++	+−−−	++++	++++	++++	++++
Dry soil+leaf mold	2	+++−	−−−−	−−−−	S−−−	+++−	−−−−	−++−	+++−	−−−−	+++−
	20	+++−	−−−−	−−−−	S−−−	+++−	−−−−	+++−	+++−	−+−−	+++−
	200	++++	−−−−	−−−−	−−−−	++++	+++−	++++	++++	+++−	++++
Moist soil	2	+++−	−−−−	−−−−	S−−−	+++−	−−−−	−++−	−−−−	−−−−	−−−−
	20	+++−	−−−−	−−−−	−−−−	+++−	−−−−	+++−	−−−−	−−−−	−−−−
	200	++++	−−−−	−−−−	−−−−	++++	−−−−	++++	++++	++±−	+++−
Moist soil+leaf mold	2	+++−	− S S−	−− S−	S− S−	+++−	− −S−	−++−	−++−	−−−−	−−+−
	20	+++−	− S S−	−− S−	S− S−	+++−	− S S S	++++	++++	−−−−	+++−
	200	++++	− S S−	−−−−	S− S S	++++	− S−−	++++	++++	−+−−	+++−
Moist soil+lime	2	+++−	−++−	−−−−	−−−−	+++−	−++−	−++−	++++	−−−−	+++−
	20	+++−	−++−	−−−−	− S S−	+++−	+++−	+++−	++++	+++−	+++−
	200	++++	+++−	+++−	− S−−	++++	++++	++++	++++	+++−	++++
Moist soil+lime+leaf mold	2	+++−	−+−−	−−−−	−−−−	+++−	−+−−	−++−	−++−	−+−−	−−−−
	20	+++−	−+−−	−−−−	− S S−	+++−	−+−−	+++−	+++−	−+−−	+++−
	200	++++	+++−	−−+−	−−−−	++++	++++	++++	++++	+++−	+++−

* From left to right, each group of entries indicates effect on bean, mustard, marigold, and barley, respectively: "+" indicates obvious toxicity; "S" shows obvious stimulation; "−" indicates no effect. Data are based on comparison with plants in control soil similarly treated except lacking 2,4-D.



FIGS. 7–10.—Test plants on May 25 in replanting of May 12. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 7, moist soil; fig. 8, moist soil + leaf mold; fig. 9, moist soil + lime; fig. 10, moist soil + lime + leaf mold.

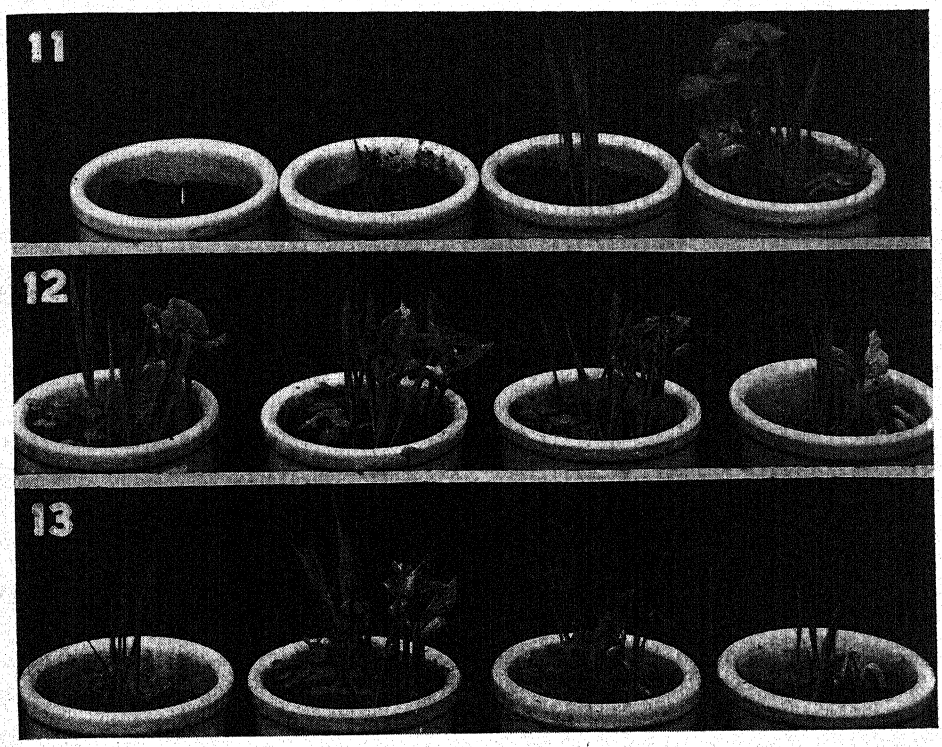
diminution of toxicity in the lots of moist soil+lime+leaf mold as shown by the plantings of May 5, July 17, and August 15 in contrast with strong persistence of toxicity in the moist soil+lime combination.

Inhibitory effects in replanted soil

As shown by the replanting on May 12 in soil which had been cropped in the greenhouse for 4 weeks, toxic effects from 2,4-D had completely disappeared except in the limed lots of soil (figs. 7–10). In the latter, some toxicity to one or more of the test species was still present at all test levels of 2,4-D (table 1, figs. 3, 4), although generally in lower degree than in the April 14 planting. The presence of partially decomposed leaf mold, however, had markedly reduced the toxic condition in the limed soil, especially in the case of marigold, and for bean in the 200-p.p.m. soil (figs. 9, 10). Some apparent stimulation of growth was evident in mustard, barley, and bean in the lots of dry soil with initial concentrations of 20 and 200 p.p.m. of 2,4-D, and also in mustard and marigold in all levels in moist soil+leaf mold (fig. 8).

A replanting on June 2 of soil which had been cropped in the greenhouse for 7 weeks showed strong inhibition in the 200-p.p.m. moist soil+lime, except in the case of barley, although the toxicity was somewhat less than on May 12. As in the May 12 replanting, the presence of leaf mold had further reduced the toxic condition in the limed soil.

In the July 15 replanting in soil which



Figs. 11–13.—Test plants on May 25 in replanting of May 19. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 11, moist soil + lime; fig. 12, top half of soil mass in planting of April 21, moist soil + leaf mold; fig. 13, bottom half of soil mass.

had been cropped in the greenhouse for $13\frac{1}{2}$ weeks, toxic effects from 2,4-D had completely disappeared in all treatments, including the limed soils. Moreover, there was some apparent stimulation to growth of all test species, in comparison with controls, in some of the lots which had been treated with 2,4-D (table 1).

In comparing the replantings of May 19 and August 16 with the replanting of May 12 (table 1), all three of which were made on soil which had been cropped in the greenhouse for 4 weeks, it appeared



FIG. 14.—Test plants on June 10 in replanting of May 19 in soil originally planted on April 21, moist soil + lime + leaf mold. *Left*, bottom half of soil mass in April 21 planting; *right*, top half.

that the May 19 replanting practically repeated the results of May 12, except in the case of dry soil+leaf mold, which remained toxic to the three dicotyledons in the 200-p.p.m. soil. In the resowing of August 16, deleterious effects of 2,4-D had apparently been more persistent than in the other comparable replantings, since there was still pronounced toxicity to one or more test species in all soil originally containing 200 p.p.m. of 2,4-D. Possibly the very high greenhouse temperatures during the August 16 test intensified the activity of plants in response to the presence of the growth-regulator, or for various reasons it had not been lost from the soil or inactivated as rapidly.

In the replanting on May 19 of the upper and lower portions of soil from pots which had been cropped in the greenhouse for 4 weeks, a strong contrast in residual toxicity between top and bottom portions, as shown by seedling emergence and later development, occurred only in the 200-p.p.m. lots (figs. 11–14). The lower half was definitely more toxic to mustard and marigold, but Red Kidney bean in this portion also showed symptoms of toxicity in misshapen leaves and yellowish-green color in the seedlings. The effect of leaf mold in diminishing the toxicity of 2,4-D in the limed soil was well shown in this planting (figs. 11, 14).

These data suggest that some of the relatively greater decrease in toxicity of the top layers may have resulted from the downward leaching of 2,4-D from the soil in the pots, in spite of the care used in watering, especially since it disappeared much less rapidly from moist stored soil. HANKS (6) and DE ROSE (4) have both shown that 2,4-D may be leached rapidly from some soils.

In conclusion, in soil lots lacking added lime, 4 weeks of cropping in the greenhouse generally sufficed to eliminate the toxicity of all the different original levels of 2,4-D. There was, however, strong residual toxicity in the limed lots in all three replantings which followed 4 weeks of cropping. Some toxicity in the 200-p.p.m. treatment persisted in the limed soil after 7 weeks of cropping, and possibly more, but disappeared completely between the replanting dates of June 2 and July 15. In each replanting test the presence of partially decomposed leaf mold had offset, to a considerable extent, the effects of the added lime in delaying the loss of

toxicity, especially in the 2- and 20-p.p.m. levels.

Discussion

The striking persistence, after cropping for 4 weeks, of the toxic effects of 2,4-D on mustard and marigold in the limed soil originally treated with 2, 20, and 200 p.p.m. of 2,4-D, and on Red Kidney bean at the 200-p.p.m. level, is regarded by the writer as the most significant result of the experiment. In an experiment on leaching 2,4-D from soil, HANKS (6) found that its inhibitory effects persisted longer in a naturally alkaline, sandy clay soil, pH 8.4, than in any other of the six soils tested. He found, however, that addition of lime to these six soils seemed to produce no significant differences in toxicity of comparable leachates as tested by a slight modification of the corn-root bio-assay developed by SWANSON (10). In confirming the results of the bio-assay, HANKS tested Red Kidney bean emergence and development and observed that the alkaline soil mentioned was the only one showing toxic effects of 2,4-D to bean at the end of 6 weeks of leaching. HANKS's experiment was based on an original 20-p.p.m. level of 2,4-D mixed in the soil in contrast to the concentrations of 2, 20, and 200 p.p.m. used in the writer's experiment.

TAYLOR (11), working with two soils, pH 7.8–7.9 and pH 6.9–7.0, found that dicotyledonous weeds were almost completely eliminated for the growing season on these medium-light field soils treated at the start of the season with 1–4 lb. of 2,4-D per acre. In general, he found that the development of bean and mustard was more strongly inhibited than that of wheat and corn in proportion to intensity of 2,4-D treatment. He further noted the possible influence of marl ($CaCO_3$) in the soil which was low in organic matter. He reported a persistence as long as 7 weeks of the toxic effects of 2,4-D applied at the rate of 4 lb. per acre. In the greenhouse the writer found that effects of 2,4-D at an initial concentration of 200 p.p.m. continued for 50 days, at least, in the case of the moist soil+lime.

HAMNER *et al.* (5), working with muck soil, reported that the toxic effects of 2,4-D mixed in it did not persist longer than 4 weeks. HANKS (6) found that the leachate from peat so treated was relatively free of toxicity, as measured by corn-root growth, 2 weeks after the incorporation of 2,4-D. In the writer's experiment the presence of large amounts of leaf mold in the soil reduced the effect of added lime in retarding the loss in toxicity.

After 18½ weeks treated soil in storage had retained most of its toxicity at all levels of 2,4-D with one exception, that of moist soil with original concentrations of 2 and 20 p.p.m. of 2,4-D. There was practically no loss of toxicity in soils stored air-dry. MITCHELL and MARTH (7) found a retention of toxicity for as long as 18 months in soil in relatively dry storage.

The more vigorous growth of some seedlings in 2,4-D–treated soil, in contrast to controls, in the replantings of May 12, June 2, and July 15 is noteworthy in many of the soil combinations. This apparent stimulation was greatest in the mixture of moist soil+leaf mold. HANKS found a stimulation of corn-root growth, in contrast with controls, in one or more of the weekly leachates from five of six soils treated with 2,4-D, the exception being the naturally alkaline soil mentioned previously. TAYLOR (11) also reported a stimulating effect on some plantings on soil previously treated with 2,4-D.

It has been demonstrated (1, 4) that germinating seeds are very susceptible to small amounts of certain growth-regulating substances, certain broadleaf species being more sensitive than some cereals. The behavior of Red Kidney bean, African marigold, and mustard in this experiment paralleled these findings for the dicotyledons, while barley showed resistance to 2,4-D in all initial plantings at 2 and 20 p.p.m. The single exception to this statement concerning barley appeared in the planting of July 17, especially on moist soil+lime, in which higher temperatures may have been an important factor in accentuating the toxic symptoms.

Modification of leaf form in bean repeated itself with surprising regularity in initial plantings in the 20-p.p.m. pots and resembled somewhat the morphogenic effects on various leaves described by ZIMMERMAN and HITCHCOCK (13) and by DEROSE (4). The appearance of Red Kidney bean seedlings in the initial plantings on soil containing 2, 20, and 200 p.p.m. of 2,4-D in this experiment closely duplicated those illustrated by ALLARD *et al.* (1).

Leaching, decomposition through action of micro-organisms in the soil, and inactivation due to adsorption or fixation by soil colloids have been suggested as factors contributing to the reduction or loss in activity of 2,4-D in soil (4, 6). The writer's experiment suggests that the status of its lime content and the organic content of soil may strongly affect the rate of loss of toxicity from 2,4-D mixed in the soil, whatever the mechanisms involved. Toxicity was also gradually diminished under some conditions of moist storage of soil in contrast with its retention in dry storage, although conditions in moist cropped soil led to a much more rapid loss of toxicity than did those in comparable moist stored soil. It is thought that reduction of toxicity in stored soil could not have resulted from leaching and that leaching was not the primary factor in the loss of toxicity in cropped soil.

Summary

1. Partially decomposed leaf mold, water, and lime were mixed with heavy black-loam soil from northern Illinois in such fashion as to yield six lots: air-dry soil; air-dry soil+leaf mold; moist soil; moist soil+leaf mold; moist soil+lime; and moist soil+lime+leaf mold. To four equal aliquots of each of the six lots, 2,4-dichlorophenoxyacetic acid (2,4-D) was mixed on April 7 at the rate of 0, 2, 20, and 200 p.p.m., respectively. All twenty-four lots were placed in storage.

2. Portions of each lot were placed in pots on greenhouse benches on April 14, April 21, May 5, July 17, and August 15. Each pot was planted with Red Kidney bean, mustard, African marigold, and barley to test by the emergence and later development of these plants the degree and persistence of toxicity from 2,4-D in the different lots of stored soils.

3. The soil originally planted on April 14 was replanted on May 12, June 2, and July 15 to test for persistence or loss of toxicity in the various treatments under cropping. Soils initially planted on April 21 and July 17 were replanted on May 19 and August 16, respectively.

4. The capacity to induce toxic symptoms was strongly persistent in soil in dry storage as measured by all initial test plantings. There was some diminution of toxicity in some lots in moist storage, although others, especially the limed ones, retained strong toxicity for 18 weeks.

5. In general, as shown by three different replantings, 4 weeks of cropping in

the greenhouse sufficed to eliminate toxicity to all test species from all soil except that which had been limed or to which 2,4-D had been added at the rate of 200 p.p.m. Seven weeks of cropping resulted in the loss of toxicity to all test species from all except the limed soil at the 200-p.p.m. level. Such long persistence of 2,4-D in this treatment suggests caution in its use in calcareous or newly limed soils. In all replantings the presence of large amounts of leaf mold strikingly reduced the degree and persistence of toxicity to one or more test species in the limed soil. Thirteen weeks of cropping resulted in complete loss of toxicity from all pots.

6. In some replantings there was more vigorous growth of seedlings in soil treated with 2,4-D than in the controls. Such stimulation was found in one or more test species in one or more replantings for twelve of the eighteen lots treated with 2,4-D.

7. Sensitivity to 2,4-D in the soil increased in the order barley, bean, mustard, and African marigold. In the initial plantings on soil containing 2 and 20 p.p.m. of 2,4-D, the first trifoliate leaves of bean characteristically showed abnormalities involving nondivergence of the leaflets or their multiplication to numbers as high as ten.

The writer wishes to express gratitude to Dr. CHARLES E. OLMSTED, under whose immediate supervision all phases of the experiment were carried on.

DEPARTMENT OF BIOLOGY
CENTRAL MICHIGAN COLLEGE OF EDUCATION
MOUNT PLEASANT, MICHIGAN

LITERATURE CITED

1. ALLARD, R. W.; DEROSE, H. R.; and SWANSON, C. P. Some effects of plant growth-regulators on seed germination and seedling development. BOT. GAZ. 107:575–583. 1946.
2. BEAL, J. M. Some telemorphic effects induced in sweet pea by application of 4-chlorophenoxyacetic acid. BOT. GAZ. 105:471–474. 1944.
3. ———. Further observations on the telemorphic effects of certain growth-regulating substances. BOT. GAZ. 106:165–178. 1944.
4. DEROSE, H. R. Persistence of some plant growth-regulators when applied to the soil in herbicidal treatments. BOT. GAZ. 107:583–589. 1946.
5. HAMNER, C. L.; MOULTON, J. E.; and TUKEY, H. B. Treatment of muck and manure with 2,4-dichlorophenoxyacetic acid to inhibit germination of weed seeds. Science 103:476–477. 1946.
6. HANKS, R. W. Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching. BOT. GAZ. 108:186–191. 1946.
7. MITCHELL, J. W., and MARTH, P. C. Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 107:408–416. 1946.
8. NUTMAN, P. S.; THORNTON, H. G.; and QUASTEL, J. H. Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant-growth substances. Nature 155:498–500. 1945.
9. PORTER, R. H. Testing the quality of seeds for farm and garden. Iowa Agr. Exp. Sta. Res. Bull. 334. 1944.
10. SWANSON, C. P. A simple bio-assay method for the determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solutions. BOT. GAZ. 107:507–509. 1946.
11. TAYLOR, D. L. Growth of field crops in soil treated with chemical growth-regulators. BOT. GAZ. 108:432–445. 1947.
12. VAN OVERBEEK, J., and VELEZ, ISMAEL. Use of 2,4-dichlorophenoxyacetic acid as a selective herbicide in the tropics. Science 103:472–473. 1946.
13. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Substituted phenoxy- and benzoic acid growth substances and relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–343. 1942.

SOME RESULTS OF CROSS-POLLINATION ON LILIUM REGALE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 584

J. M. BEAL

One of the most popular of our garden lilies is *Lilium regale* Wilson, commonly known as the regal lily. Because of the many desirable characters which it possesses, numerous attempts have been made at hybridizing it with other species, but they have generally been unsuccessful when *L. regale* was used as the seed parent. Although the application to the stigmas of *L. regale* of pollen from almost any species of *Lilium* may result in the production of a greater or fewer number of viable seeds, the progeny derived through such pollinations, almost without exception, have shown no evidences of paternal inheritance.

Partly in an effort to learn why this result was obtained, a large number of cross-pollinations have been made during the last decade on *L. regale*, both in our local garden and at Wychwood, Lake Geneva, Wisconsin. These experiments were begun in 1936 and have been continued each season, using pollen from *L. amabile*, *L. browni*, *L. canadense*, *L. candidum*, *L. concolor*, *L. martagon*, *L. pumilum*, *L. speciosum*, *L. superbum*, *L. umbellatum*, and *L. willmottiae* (*L. davidi* var. *willmottiae*) as well as from a few hybrids including *L.* × Backhouse, *L.* × Maxwill, *L.* × *elegans*, and *L.* × *testaceum*. In some cases as few as four or five seeds per capsule have developed after pollination, while in others, notably when the pollen of *L. candidum* was used, practically all the ovules developed into mature viable seeds. There was also marked seasonal variation in the quantity of seeds set. For example, the summers of 1936 and 1938 were unfavorable, and comparatively few seeds developed, whereas during 1937 and 1946 heavy sets of seeds developed in at least some capsules from almost all the pollinations.

Seeds resulting from the cross-pollinations were planted in flats in the greenhouses in late November or early December of each year. Usually a high percentage of germination was obtained. The seedlings were allowed to remain in the flats until late May or early June following, when they were transplanted to the garden. For a few years the small bulbs resulting from this first season's growth were sent to the Willamette Valley of Oregon for further development because of the more suitable climatic conditions prevailing there. Owing to transportation difficulties which developed during the war, however, the practice was discontinued after 1941, and the seedlings have since been grown in our local garden.

Both here and in Oregon some of the seedlings flowered in the third season and most of them in the fourth. Regardless of the pollen which had been used on the seed parent, all first-generation progeny appeared to be pure *L. regale*, with no greater variability in flower color or size, leaf form, and size or height of stems than occurred in the progeny of selfed *L. regale*.

A number of flowers from a part of nearly all the first-generation crosses were self-pollinated. Generally the capsules set an abundance of seeds, approxi-

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

mating that of selfed *L. regale* closely in quantity. The seeds showed high viability, and their seedlings were as vigorous as those of *L. regale* seedlings. The time required for flowering in the supposed F_2 plants was the same as for the seedlings of the first-generation crosses or for seedlings from selfed *L. regale*. These supposed F_2 plants still appeared to be pure *L. regale*, showing no segregation and no indication whatever of hybridity.

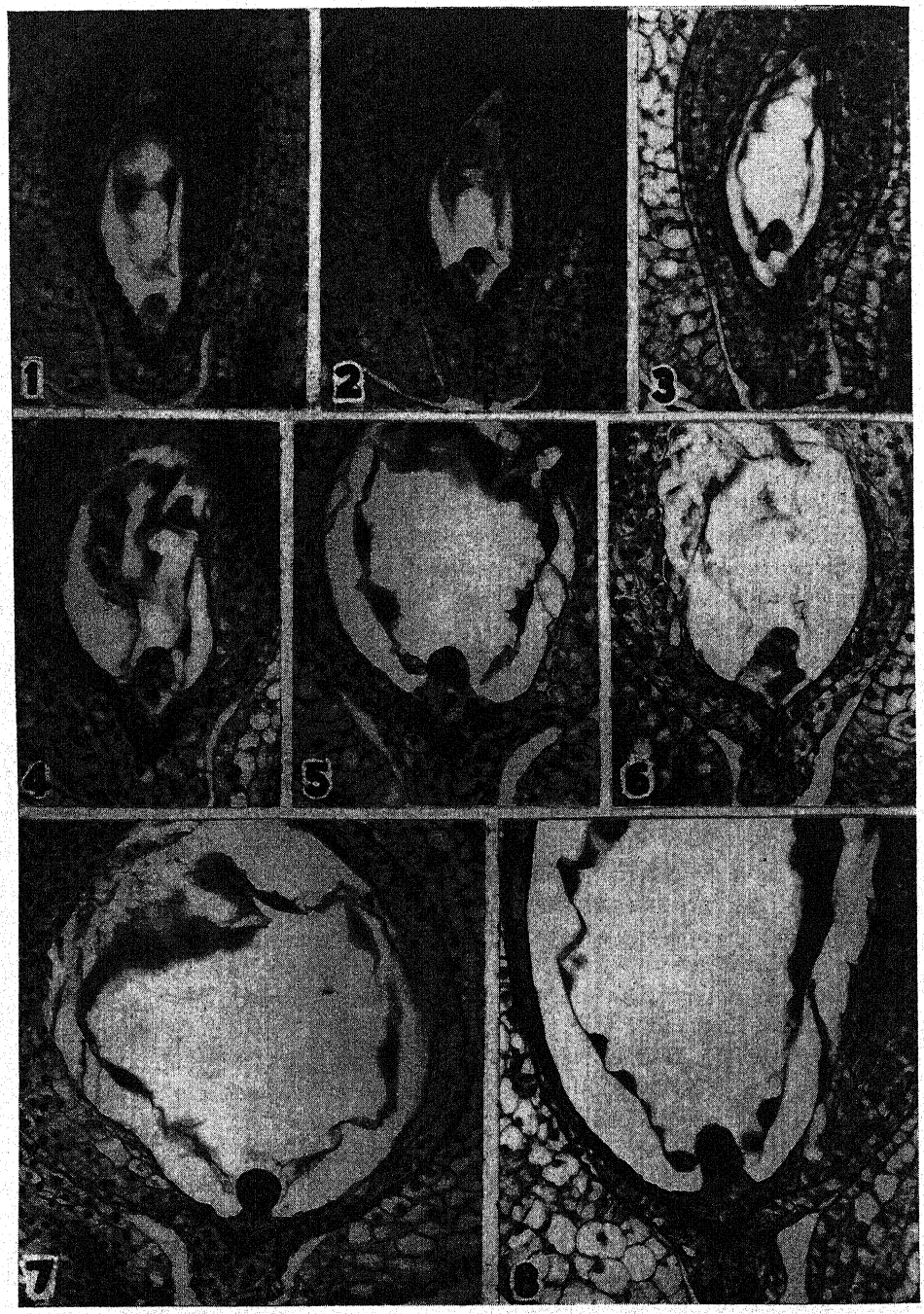
For several years, too, Professor RONALD BAMFORD and certain of his graduate students at the University of Maryland have been working on the same problem. In both personal conversation and correspondence Professor BAMFORD has informed me that their results and observations are in complete agreement with mine. The photomicrographs, which constitute a part of the present paper, were sent to him in order that he might compare them with their preparations.

Several hundred smear preparations of microsporocytes from the first-generation crosses have been made, stained according to the gentian violet–iodine method, and compared with similar smears of microsporocytes of *L. regale*. No observable differences in pairing relations, chiasma frequency, or disjunction have been detected. Neither lagging nor other detectable differences could be observed. In short, meiosis in the progeny resulting from the cross-pollinations showed no peculiarities which would serve to distinguish them from the progeny of selfed *L. regale*.

Ovaries were collected at appropriate intervals during several of the years from both self- and cross-pollinated plants, cut transversely into segments usually 5–8 mm. long, fixed in NAVASHIN's solution, handled according to the tertiary butyl-alcohol method, and sectioned, according to age, at thickness ranging from 20 to 35 μ. Megasporogenesis and megagametophyte development in *L. regale* follow the *Fritillaria* type, according to the results of COOPER (1), and my observations are in complete agreement with his report.

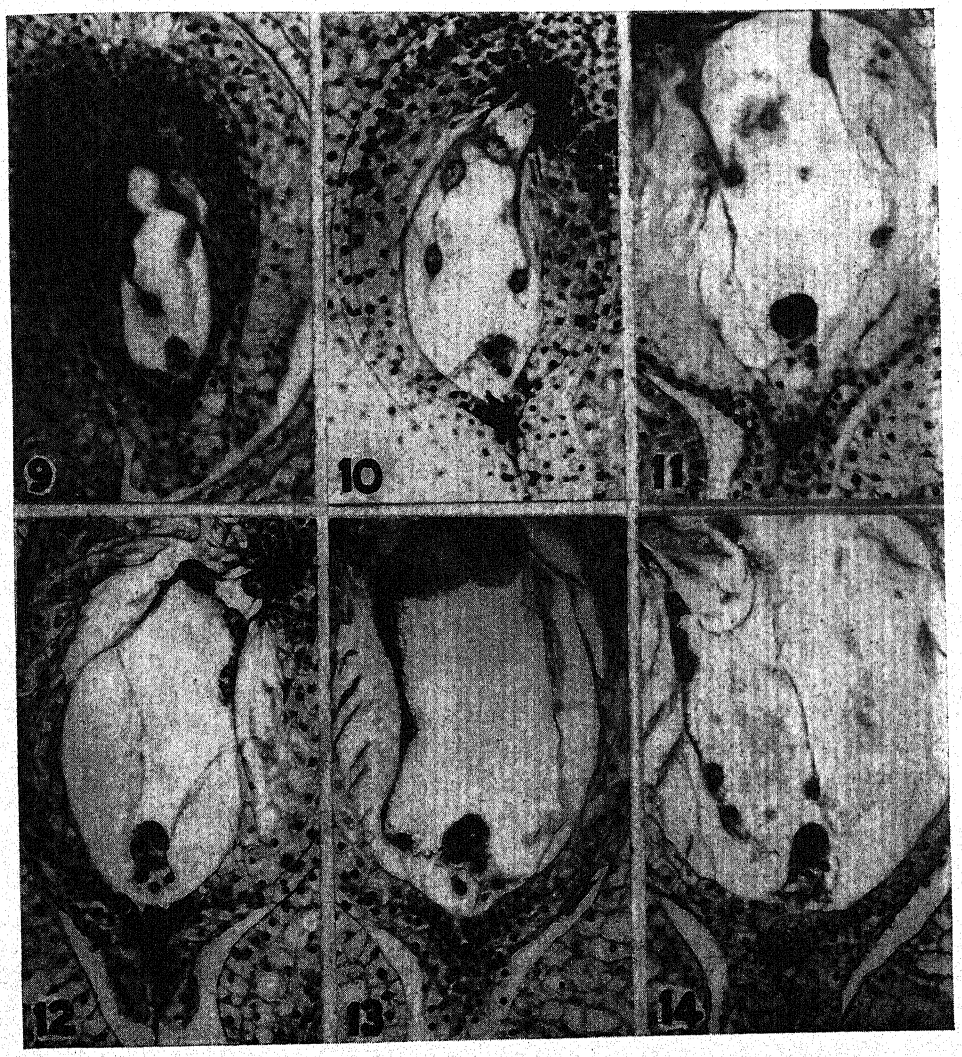
In self-pollinated plants the pollen tubes reached the ovules at the distal end of the ovary in approximately 100 hours, while to reach those at the proximal end sometimes required as long as 120 hours. The time may vary, however, as a result of variable environmental conditions so that, when especially favorable conditions prevail, the ovules nearest the stigmas may show the pollen tubes in their micropyles as early as 96 hours or, conversely, when conditions are not favorable, it may require 120 hours or more. Rather large numbers of ovules were seen which showed the pollen tubes shortly after they had passed through the micropyles with the two microgametes already discharged into the megagametophyte. The microgametes, when first discharged, are elongated and vermiform, as has been noted frequently by other investigators. Various stages in the double-fertilization process have been observed which, in the main, differ little from previous descriptions (3, 4, 5).

At 188 hours following self-pollination the primary endosperm nucleus had divided once, while the zygote nucleus remained undivided (fig. 1). At 216 hours there were four nuclei in the endosperm and a two-celled embryo (fig. 2). At the side of this embryo was a second structure which possibly might be a twin embryo derived from one of the synergids, as has been reported for *Lilium* by COOPER (2). A similar condition has been observed in only a few other sections of the self-pollinated ovaries and in none of those from the cross-pollinated



FIGS. 1–8.—Stages in development of embryo and endosperm in self-pollinated *Lilium regale:* fig. 1, 188 hours, with undivided zygote nucleus and two-nucleate endosperm; fig. 2, 216 hours with apparently twin embryos and at least an eight-nucleate endosperm; fig. 3, 268 hours, with embryo and endosperm only little more developed; fig. 4, 312 hours, with embryo sac starting to enlarge; fig. 5, 360 hours, showing increased volume of endosperm in enlarged embryo sac; fig. 6, 408 hours, with only slight advances over preceding; fig. 7, 456 hours, similar to preceding; fig. 8, 485 hours, endosperm greater in volume and number of nuclei, embryo relatively little farther advanced.

ones. At 268 hours the endosperm appeared to possess at least sixteen nuclei and the embryo several (fig. 5). The volume of the embryo sac next increased noticeably as the volume and number of cells of the endosperm increased (figs. 4–6), but with proportionately less rapid increase in volume of the embryo. The growth of the embryo does not keep pace with that of the endosperm (fig. 7, 456 hours; fig. 8, 485 hours). During the period of approximately 500–900 hours following fertilization, the embryo remains relatively small and little differentiated, according to some other material examined but not reported on herewith, while the endosperm continues to develop rapidly.



FIGS. 9–14.—Stages in development of embryo and endosperm in *L. regale* pollinated with pollen of *L. candidum:* fig. 9, 288 hours, similar to fig. 2; fig. 10, 360 hours, similar to fig. 3; fig. 11, 432 hours; fig. 12, 456 hours, similar to fig. 6; fig. 13, 468 hours, similar to fig. 7; fig. 14, 492 hours, similar to fig. 8.

Although the earliest stages from cross-pollinated plants have been found at 288 hours after pollination, the evidences of pollen-tube entrance through the micropyle were still clear (fig. 9). The endosperm development is comparable with that in selfed plants (fig. 3), but embryo development is possibly a little slower. At 360 hours (fig. 10) development of both embryo and endosperm is comparable with that in selfed plants (cf. fig. 5). The embryo sac next increases markedly in volume (figs. 11–14), just as in the self-pollinated plants, and the appearance of embryos and endosperm is strikingly similar in the two series, with about equal development at the same time intervals. So far as it has been possible to determine, there are no recognizable differences in development of endosperm and embryo in self- and cross-pollinated plants.

Additional studies have been carried out on the cross-pollinated plants, with collections having been made at 1- or 2-day intervals for more than 50 days after pollinations. The ovules, which were nearly mature seeds at that time, contained essentially fully developed embryos. Many of the capsules were as well filled as those on self-pollinated plants. No recognizable differences whatever could be observed.

These studies have so far given neither information nor real clues to the reason why the progeny resulting from the cross-pollinations show no evidences of hybridity. It is planned to continue the experiments during the summer of 1947 and to investigate materials at some of the time intervals following pollinations which are not covered in the present report.

Summary

1. During the last decade cross-pollinations have been made on *Lilium regale* with pollen from more than a dozen species of lilies. Viable seeds have developed in some capsules following all the cross-pollinations. The seedlings resulting from these seeds reach flowering size during the third and fourth seasons following planting, but among several thousand of the supposed F_1 hybrids no evidences whatever of the pollen parent have been manifested; they closely resemble the seed parent in all visible characters. These seedlings show no greater variability than that exhibited by seedlings resulting from self-pollination.

2. Smear preparations of microsporocytes from the supposed F_1 hybrids showed no differences in meiotic behavior when compared with microsporocytes of *L. regale*. Following cross-pollination, the pollen tubes entered the ovules by way of the micropyles, and both embryos and endosperm developed at approximately the same rate and in the same manner as in selfed *L. regale*. No explanation has so far been found for this unusual behavior.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. COOPER, D. C. Macrosporogenesis and the development of the embryo sac of *Lilium henryi*. BOT. GAZ. **97**:346–355. 1935.

2. ———. Haploid-diploid twin embryos in *Lilium* and *Nicotiana*. Amer. Jour. Bot. **30**:408–413. 1943.

3. GUIGNARD, L. Sur les anthérozoides et la double copulation sexuelle chez les végétaux angiosperms. Compt. Rend. Acad. Sci. (Paris) **128**:864–871. 1899.

4. NAVASHIN, S. Resultate einer Revision der Befruchtungsvorgänge bei *Lilium Martagon* und *Fritillaria tenella*. Bull. Acad. Imp. Sci. (St. Petersbourg) **9**:377–382. 1898.

5. ———. Neue Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*. Bot. Centralbl. **77**:62. 1899.

DEVELOPMENTAL SEQUENCE OF EMBRYO AND ENDOSPERM IN APOMICTIC AND SEXUAL FORMS OF POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

It is well established that the proembryo may consist of several cells before the first division of the fusion nucleus in apomictic forms of Kentucky bluegrass, *Poa pratensis* L. The significance of some of the figures of the developing female gametophyte of this species published by ANDERSON (3) has only recently been recognized. TINNEY (5) found that pollination was unnecessary to induce the formation of proembryos but that without pollination no endosperm was formed. This was recently substantiated by ÅKERBERG (1, 2), whose demonstration of the necessity of fertilization of the polar nucleus for endosperm formation was based upon an extensive series of emasculations. The sizes of the complements of chromosomes in this tissue could be accounted for only by assuming that fertilization had occurred. The latter observation was confirmed by NIELSEN (4).

Opportunity was offered for making some additional observations on this phenomenon during the course of cytological studies of the species. It appears desirable to record briefly some of the more salient facts that have been brought to light.

Material and methods

Kentucky bluegrass plants flower in the early morning at Madison, Wisconsin. Anthers are exserted and the pollen is mostly shed before 6:00 A.M. on bright days of late May.

Material was gathered from known sexual and apomictic plants growing in the breeding nurseries (4). From each of two sexual and two apomictic plants all spikelets of panicles without lowermost florets at anthesis were removed on the first day these plants flowered. The remaining spikelets, those wherein the lowermost florets were at anthesis, constitute the material upon which this study was based. These were fixed in Sax's modification of Navashin's fluid. The first fixation was made at 9:00 A.M., and subsequent ones at 24, 48, 120, 216, and 264 hours later. The material was transferred to 70% alcohol the following day. Dehydration was by the usual alcohols, and the imbedded material was sectioned at 15 μ. Iodine-ripened haematoxylin was used for staining.

Observation was limited to the lowermost floret of the spikelet in compiling the accompanying data, with one exception. Data for florets 20 hours before anthesis were derived from the second floret of the spikelets of the first fixation. This was based upon the assumption that this floret would have been at anthesis the day after fixation had it remained upon the plant.

There is usually considerable delay in the migration of the polar nuclei as well as in the division of the fusion nucleus. Several arbitrary values were therefore set for the different positions of the nuclei of the developing female gametophyte. These are illustrated in

[1] Results of co-operative work between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, Madison.

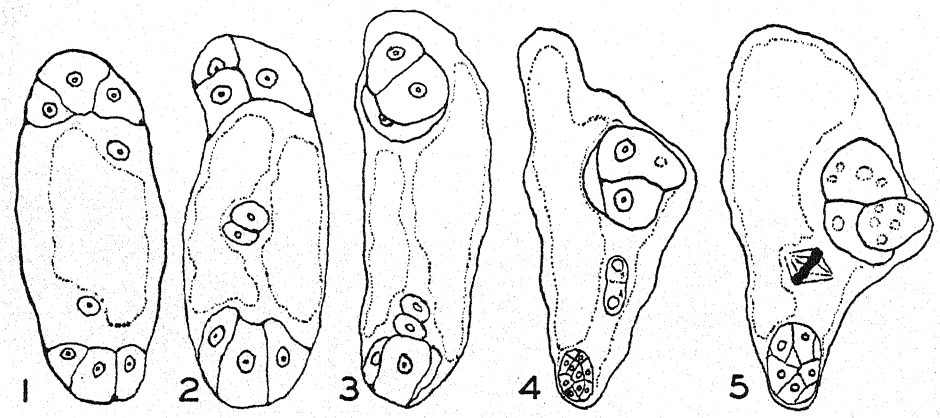
figures 1–5. It is recognized that there may be deviations from these arbitrary classes, but for purposes of tabulation this affords a method of establishing comparative data.

Results

The observations are tabulated in table 1. The developmental sequence is markedly different in the two types of plants during the first 2–3 days following anthesis. A proembryo averaging eight to sixteen cells was present in the apomictic plant a day before anthesis. Only two observations were made at this stage for the sexual plants. The polar nuclei had migrated toward the center of the embryo sac in each case.

Four hours after anthesis the proembryo in embryo sacs of apomictic plants averaged from sixteen to thirty-six cells in size, the polar nuclei being in positions of from 1 to 3. Florets of sexual plants collected at comparable intervals showed varying stages of development ranging from a four-nucleate endosperm (one instance) to stages of fusion of the polar nuclei in a position adjacent to the zygote and proembryo (five instances). In two embryo sacs the polar nuclei were lying in the central position. The proembryos of embryo sacs of the sexual plants averaged about a third the size of those in their apomictic counterparts.

In no instances were endosperm nuclei observed in material taken from the apomictic plants 28 hours after anthesis. Nine of twenty comparable embryo sacs of sexual plants had developed free-nucleate endosperm, the number of nuclei averaging eight and sixteen.



FIGS. 1–5.—Stages of development of embryo sac in *Poa pratensis*. Fig. 1, polar nuclei migrating toward center of embryo sac. Fig. 2, polar nuclei lying near center of embryo sac. Fig. 3, polar nuclei lying adjacent to egg. Fig. 4, polar nuclei migrating toward antipodals. Fig. 5, first division of polar fusion nucleus just below antipodals. Figures are not drawn to scale.

Two days after anthesis the endosperm tissue in the florets of the apomictic plants had developed greatly, the average number of nuclei observed being 116 and 236. The endosperm nuclei of the sexual plants averaged 13 and 16 at the corresponding stage. The proembryos averaged 10 and 12 cells in the same florets and were approximately one-fourth as large as those in the embryo sacs of the apomictic plants with which they are compared.

Five days after anthesis, and subsequently, there is no clear-cut difference between the two types of plants in em-

bryo and endosperm development. The endosperm is partially cellular and the undifferentiated embryo rather large. Differentiation of the latter is well under way at 9 days, as is food storage in the endosperm. Eleven days after anthesis the endosperm is in dough and, to all appearances, the embryo differentiated into its several tissues.

The slow initial growth of the proembryo in florets of the sexual plants during the first 48 hours following anthesis is notable as well as the apparent rapid acceleration during the next 3 days. These

TABLE 1

DEVELOPMENT OF EMBRYO IN RELATION TO THAT OF ENDOSPERM IN SEXUAL AND IN APOMICTIC PLANTS OF *Poa pratensis**

Plant	Time (hours) from anthesis	Florets examined	Character of zygote or proembryo†		Position of polar nuclei					Average number of endosperm nuclei or character of endosperm
			Zygotes observed	No. cells in proembryo	1	2	3	4	5	
	Sexual									
70-11	4	3	.....	11.0	....	2	1	....	....	.....
	28	13	.....	11.7	....	1	....	7	1	8.0
	52	8	.....	10.5	....	....	....	4	....	16.2
	124	16	.....	Massive	....	....	....	....	....	259 to cellular at base
	220	13	.....	Differentiating	....	....	....	....	....	Cellular and food materials stain densely
	268	16	.....	Differentiated	....	....	....	....	....	In dough
72-16	— 20	2	2	.....	....	2	....	....	....	.....
	4	6	.....	6.6	....	....	5	....	....	4.0
	28	7	.....	26.0	....	....	1	1	....	16.2
	52	5	.....	12.5	....	....	1	1	....	13.3
	124	6	.....	44.4	....	....	....	....	....	Free nucleate to cellular
	220	6	.....	Differentiating	....	....	....	....	....	Cellular
	268	16	.....	Differentiated	....	....	....	....	....	In dough
	Apomictic									
72-5	— 20	4	2	8.0	2	1	1	....	....	.....
	4	9	2	36.1	1	4	4	....	....	.....
	28	14	.....	23.7	2	2	3	3	3	.....
	52	10	.....	45.2	....	2	....	....	....	116 to cellular at base
	124	7	.....	85.2	....	....	....	....	1	Cellular at base
	220	7	.....	Differentiating	....	....	....	....	....	±Cellular to cellular
	268	14	.....	Differentiated	....	....	....	....	....	In dough
72-14	— 20	8	.....	14.2	....	6	2	....	....	.....
	4	12	.....	17.4	....	10	2	....	....	.....
	28	2	.....	29.5	....	....	....	....	2	.....
	52	4	.....	36.3	....	....	....	....	1	236 to cellular at base
	124	4	.....	52.0	....	....	....	....	....	±Cellular
	220	18	.....	Differentiating	....	....	....	....	....	Cellular
	268	10	.....	Differentiated	....	....	....	....	....	In dough

* Progenies of plants 72-5 and 72-14 contained 0 and 8.3%, respectively, offtype plants; and those of plants 70-11 and 72-16 were 100% aberrant, no two plants being morphologically alike.

† Zygote includes unicellular members of embryo sacs.

are evident from a comparison of the developmental sequence in sexually and apomictically reproducing types. The late division of the fusion nucleus in the embryo sacs of the apomictic plants is fully in accord with earlier observations. There is published evidence that pollination is necessary for endosperm formation, and, without this being effected, the ovule aborts. There is evidence also from the chromosome numbers present in the endosperm tissue of apomictic plants that fertilization must have taken place to account for the sizes of the complements present. Although this evidence has been established, it is difficult from the present data to arrive at a satisfactory conclusion that accounts for the long delay from the time of pollination to the onset of formation of endosperm in the apomictic plants. The data presented indicate that fertilization to form the polar fusion nucleus could hardly have taken place less than 24 hours following anthesis. Usually it is not prior to this time that the polar nuclei are in intimate association with one another. When these observations are considered with reference to the sequence of events in the sexual plants, it becomes evident that the two types of plants differ also in respects other than those determinable from cytological study. Other techniques than those employed will apparently have to be administered to extensive materials before the underlying causes can be definitely determined.

Summary

The comparative development of the embryos and endosperms was examined in two plants each of known apomictic or sexual reproductive tendencies. The development of the embryo is well under way before the first division of the fusion nucleus in apomictic plants of *Poa pratensis*. In sexual individuals the endosperm development is in advance of that of the zygote or proembryo during the first 2 days following anthesis. Five days after pollination, and subsequently, the development of both embryos and endosperm in the two differently reproducing types of plants is essentially parallel, and the two types are then indistinguishable from each other.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ÅKERBERG, E. Cytogenetic studies of *Poa pratensis* and its hybrid with *Poa alpina*. Hereditas 28:1–126. 1942.
2. ———. Further studies of the embryo and endosperm development in *Poa pratensis*. Hereditas 29:199–201. 1943.
3. ANDERSEN, A. M. Development of the female gametophyte and caryopsis of *Poa pratensis* and *Poa compressa*. Jour. Agr. Res. 34:1001–1017. 1927.
4. NIELSEN, E. L. Cytology and breeding behavior of selected plants of *Poa pratensis*. BOT. GAZ. 106:357–382. 1945.
5. TINNEY, F. W. Cytology of parthenogenesis in *Poa pratensis*. Jour. Agr. Res. 60:351–360. 1940.

RESPONSE OF GUAYULE TO ALTERNATING PERIODS OF LOW AND HIGH MOISTURE STRESSES

H. M. BENEDICT, W. L. McRARY, AND M. C. SLATTERY[1]

Introduction

The yield of rubber per acre obtained from guayule is determined by the amount of rubber-bearing tissue present and its rubber content. Results to date (1, 2, 4, 7, 9, 10) have indicated that the rubber-bearing tissues are produced during periods of active growth but that their rubber content increases during periods of limited growth caused by drought, low temperatures, and nutrient deficiencies. This raised the question as to which method would yield the most rubber: (*a*) to allow the plants to grow vigorously for short periods of time and then to subject them for short periods to conditions favorable for building-up of rubber but unfavorable for growth, or (*b*) to allow them to grow actively for a long period, followed by a single period of conditions favorable for rubber formation. In other words, was it necessary, to obtain large yields, for the shrub to build up its rubber content as it grew—and, if so, how long should the periods of active or limited growth last—or could a large shrub be produced first which would accumulate rubber later?

This paper presents results obtained at Salinas, California, from studies on growth and carbohydrate, resin, and rubber content of guayule subjected to alternating periods of abundant moisture and drought for different lengths of time. The data, in addition to yielding some information on the above points, also indicate the changes in carbohydrates and resins induced by the various drought conditions.

[1] Physiologist, Associate Physiologist, and Assistant Physiologist, respectively, Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Department of Agriculture, Salinas, Calif. The present address of Dr. McRARY is University of California, Santa Barbara, Calif.

Methods

Nursery plants seeded in the spring of 1943 were dug the first week in March, 1944, and were carefully selected for uniformity of crown diameter between $\frac{5}{32}$ and $\frac{6}{32}$ inch. All leaves and any stem tissues more than 2 inches above the crown were pruned off. Large wooden boxes 14 × 14 inches square and 20 inches deep were filled to a depth of 3 inches with coarse gravel to allow for drainage and then to within an inch of the top with soil consisting of a mixture of two parts of soil (Chualar loam), one part of leaf mold, and one-half part of sand by volume. The plants were set in these boxes on March 6, 1944, one plant per box. Following a period of 2 months in the greenhouse at 60°–70° F., they were placed outside until September 27. Only 0.62 inch of rain fell during this outdoor period. From the time of return to the greenhouse until the final sampling on May 6, 1945, the plants grew at a temperature between 70° and 80° F.

Four different treatments were applied: (A) plants grown continuously with abundant water (low moisture stress); (B) plants grown for 2 months under low moisture stress followed by 2 months of drought (high moisture stress), with this cycle repeated until the end of the experiment—a total of four wet and three dry periods; (C) plants grown for

4 months under low moisture stress followed by 4 months under high moisture stress, with this cycle repeated until the close of the experiment; (D) plants grown for 10 months under low moisture stress followed by 4 months under high moisture stress.

The experiment was designed to last 16 months, but, because of the discontinuance of the project, the final harvest was made 2 months ahead of schedule. Thus, the final results on treatment B were obtained at the close of a wet, rather than a dry, period, and those on treatment C were taken after a drought period of 2, instead of 4, months.

The moisture stresses or degrees of soil drought to which the plants were subjected were indicated by the electrical resistance method described by BOUYOUCOS and MICK (3). The plaster of Paris blocks were buried 10 inches from the surface of the soil in several of the boxes. The resistance of the blocks with moisture content of the soil at field capacity was about 300 ohms; at the wilting-point, between 450,000 and 600,000 ohms (5, 8). When the plants were grown in the presence of abundant soil moisture (low moisture stress), it was endeavored to maintain the resistance of the blocks between 300 and 800 ohms, although on occasion the moisture stress became higher and the resistance rose to as high as 3,000 ohms. However, under the latter condition the plants never showed any signs of wilting even in the middle of the day. When the plants were grown under conditions of high moisture stress, the procedure was similar to that recommended by HENDRICKSON and VIEHMEYER (6). The soil was allowed to dry until the resistance of the blocks reached approximately 250,000 ohms. Then enough water was added to reduce the resistance to about 300 ohms (field capacity of the soil), and the soil was again allowed to dry until the resistance reached 250,000 ohms. This cycle was repeated throughout the dry period.

The amount of water required to cause a drop in resistance from 250,000 to approximately 300 ohms was between 9 and 12 liters for each box of soil. The logarithms of the resistances in ohms of the blocks in treatments A, B, and C from November 6 to May 6 are shown in figure 1. The resistance curve in treatment D was the same as in treatment A until January 6, then it equaled that of B and C during their drought periods. The curve of treatment A is the mean of three replications, treatment B of five, and treatment C of two.

Plants from the various treatments were harvested at the end of each period of moisture stress. Thus plants of treatments A and B were harvested every 2 months; of treatment C, every 4 months; and of treatment D, after 10 and 14 months. Five replications of three plants each were harvested on each date. The various determinations were made on each replication. The treatments and replications were arranged in a randomized block design, the randomization scheme being taken from TIPPETS' *Random Sampling Numbers* (12). Analyses of variance were carried out on the data obtained.

In harvesting the plants a steel bar was driven into the soil about 2 or 3 inches from the main tap root. Leverage was then applied so as to lift the plant out of the ground. This is an undesirable way to harvest the roots as the many small rootlets break off and are left behind, and only the main tap roots and the larger lateral roots are obtained. Tests indicated that on a dry-weight basis about 15–20% of all roots present were left behind by this manner of har-

vesting. When the data on the roots are being considered, this source of error must be taken into account. The portions of the plants thus recovered were comparable with those recovered by methods of digging in the field, and the data are thus of value in indicating results that would have been obtained from

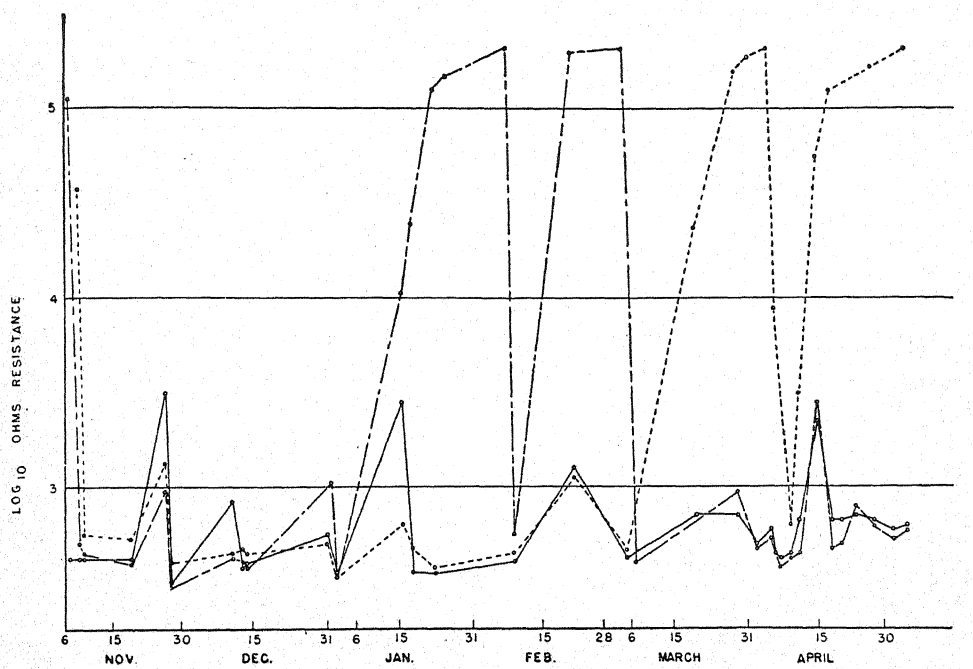


FIG. 1.—Moisture stress in different treatments from November 6 to May 6 expressed as $\log^{10}$ of resistance of Bouyoucos blocks in ohms. Value of 2.48 represents field capacity; 5.65–5.78 the wilting-point. Treatment A, solid line; treatment B, broken line; treatment C, dotted line.

field plantings grown under similar climatic and edaphic conditions.

After the plants were removed, the roots, stems, and leaves were separated. The fresh weight, percentage moisture, and dry weight of these fractions of each replication of each treatment were determined separately. The carbohydrate, rubber, and resin contents of these plants were also determined.

The percentage moisture and dry weights were obtained by drying aliquots of the fresh material in a ventilated dryer at 65° C. for 48 hours. The carbohydrate fractions, free sugars, levulins, and inulin were analyzed according to the method described by TRAUB and SLATTERY (15), and pentosans by the method of MCRARY and SLATTERY (11). Rubber and resins were determined by the turbidimetric method described by TRAUB (13).

Results

EFFECT ON DRY WEIGHTS

The most pronounced effect of the treatments was found in the weight of the leaves (table 1). High moisture stress caused a reduction in leaf weight. On the addition of soil moisture, the leaf weight never increased to that of plants grown under continuous low moisture stress.

The increases in dry weights of the roots and stems in treatment C and D (see also fig. 2) were limited by high moisture stress but to a lesser degree than those of the leaves. The first dry period limited the increase in weight of the roots and stems of the plants in treatment B. After the first cycle, however, dry weights of the stems at the end of each period of high stress in this treatment were not significantly different from those of treatment A but were significantly lower at the end of the periods of low moisture stress. These results on the stem growth of plants in treatment B cannot be accounted for, but they seem to be related to the proportion of leaves to stem at each change of moisture stress coupled with the relative short duration of the high stress period.

The greatest differences in dry weights were not between treatments but between dates. The proportionate increases were greater during the period from March to September than from September to March regardless of treatment. The enormous increase in dry weight between March and May, 1945,

TABLE 1

DRY WEIGHT PER PLANT (GRAMS) OF GUAYULE GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	2.32	9.71	21.40	29.20	33.49	43.41	70.23
B	2.32	*5.90*	18.41	*15.75*	25.03	*23.49*	54.68
C	2.32	9.71	.....	*15.37*	.....	33.48	*27.43*
D	2.32	.....	.....	.....	33.49	.....	*27.11*
Difference required $P<0.05$	.....	2.19	2.12	2.24	1.85	5.61	4.38
Stems:							
A	1.32	4.27	17.23	23.47	29.12	36.87	63.64
B	1.32	*3.34*	14.30	*23.59*	26.54	*35.01*	54.81
C	1.32	4.27	.....	*20.02*	.....	28.32	*49.97*
D	1.32	.....	.....	.....	29.12	.....	*40.93*
Difference required $P<0.05$	.....	0.66	1.98	3.19	1.78	3.44	5.56
Roots:							
A	1.29	4.08	7.62	9.04	12.50	13.25	18.18
B	1.29	*3.67*	5.54	*8.75*	10.60	*11.07*	17.32
C	1.29	4.08	.....	*8.37*	.....	11.24	*14.04*
D	1.29	.....	.....	.....	12.50	.....	*11.84*
Difference required $P<0.05$	.....	*	0.91	*	1.52	1.69	2.46
Total:							
A	4.93	18.06	46.25	61.71	75.11	93.53	152.05
B	4.93	*12.91*	38.25	*48.09*	62.17	*69.57*	126.81
C	4.93	18.06	.....	*43.76*	.....	73.04	*91.44*
D	4.93	.....	.....	.....	75.11	.....	*79.88*
Difference required $P<0.05$	.....	3.85	4.63	4.19	5.15	5.93	10.40

* Not significant.

is of interest as it occurred in all treatments but not to such an extent in dry treatments as in wet. As the available moisture and temperature were varied over the same ranges during both periods of the year, these results emphasize the importance of some other factor associated with season, such as hours of sunlight or daylength, on the growth of guayule.

At the close of the experiment the plants in treatment A had the greatest amount of rubber-bearing tissue (stems and roots) and those in treatment D the least, with weights of the plants of treatments B and C falling in between (fig. 2).

Carbohydrate content

The percentages of free sugars, levulins, and inulin on the various harvest dates are shown in table 2. Of these water-soluble carbohydrates, only the content of levulins in the stems and roots showed any marked response to the treatments. During periods of high moisture stress the levulin percentage increased; during periods of low moisture stress following a high moisture stress it decreased. The percentages of free sugars and inulin were always small and did not vary consistently with treatment.

To determine whether the reductions

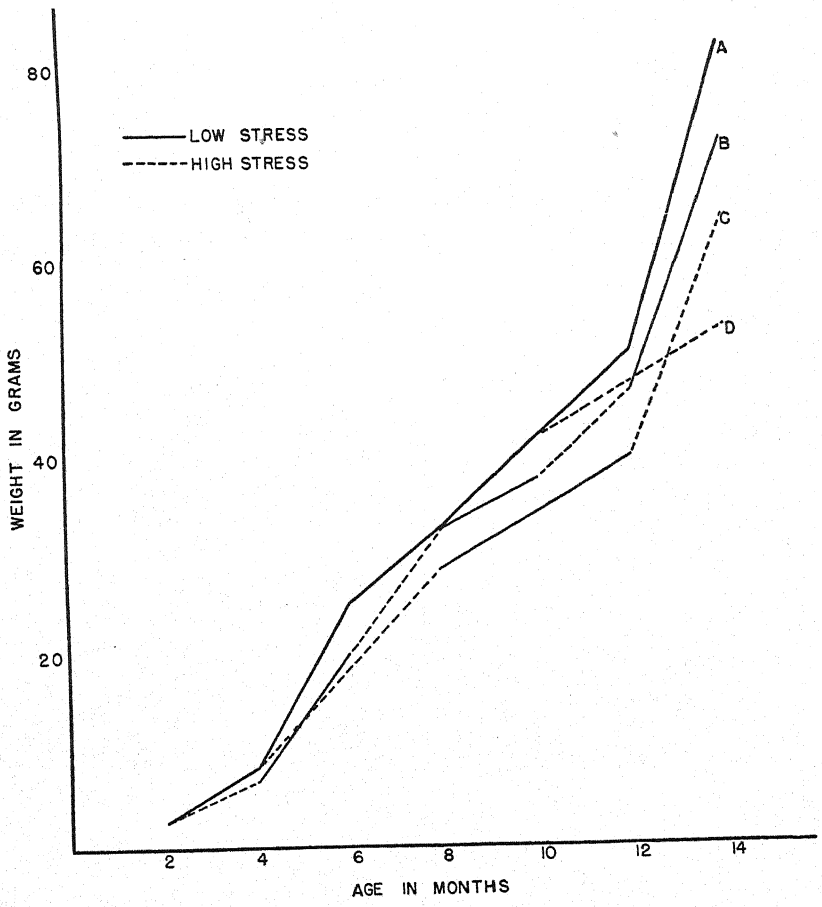


Fig. 2.—Dry weight per plant of stems and roots in different treatments

in percentage of levulins during periods of active growth following periods of slow growth represented a true loss, the grams per plant were calculated for each harvest date. These results, combining the amounts in roots and stems, have been plotted in figure 3, along with the values for free sugars, inulin, and total water-soluble carbohydrates. They show that the absolute levulin content increased markedly under conditions of high moisture stress and that the levulins pre-

TABLE 2

WATER-SOLUBLE-CARBOHYDRATE CONTENT (PERCENTAGE BASIS) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Free sugars*						
Leaves:							
A	0.48	1.10	0.91	0.41	0.42	0.41	0.65
B	.48	*0.69*	1.01	*.51*	.33	*.43*	.90
C	.48	1.10	.....	*.46*	.....	.37	*.71*
D	.48	.....	.....	.....	.42	.....	*.79*
Stems:							
A	.65	0.71	0.39	.36	.39	.30	.37
B	.65	*0.71*	0.41	*.60*	.34	*.55*	.33
C	.65	0.71	.....	*.67*	.....	.26	*.62*
D	.65	.....	.....	.....	.39	.....	*.56*
Roots:							
A	.55	0.56	0.29	.34	.31	.37	.49
B	.55	*0.77*	0.34	*.44*	.32	*.51*	.46
C	.55	0.56	.....	*0.47*	.....	0.33	*.42*
D	0.55	.....	.....	.....	0.31	.....	*0.41*
	Levulins*						
Leaves:							
A	0.46	0.85	1.42	0.59	0.49	0.48	0.57
B	0.46	*0.69*	1.61	*0.54*	0.48	*0.38*	0.53
C	0.46	0.85	.....	*0.66*	.....	0.40	*0.75*
D	0.46	.....	.....	.....	0.49	.....	*0.57*
Stems:							
A	1.30	4.75	3.26	1.91	2.44	1.80	1.26
B	1.30	*7.82*	3.07	*5.25*	1.88	*3.47*	0.89
C	1.30	4.75	.....	*5.60*	.....	1.33	*5.52*
D	1.30	.....	.....	.....	2.44	.....	*4.71*
Roots:							
A	2.37	5.24	3.25	2.13	2.56	2.73	1.20
B	2.37	*11.07*	2.95	*5.78*	2.78	*5.85*	0.61
C	2.37	5.24	.....	*6.29*	.....	2.25	*6.14*
D	2.37	.....	.....	.....	2.56	.....	*6.61*

* Difference required for significance ($P < 0.05$) between means: free sugars, 0.22; levulins, 0.78; inulin, 0.19.

TABLE 2—*Continued*

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Inulin*						
Leaves:							
A	0.05	0.33	0.52	0.42	0.42	0.36	0.41
B	.05	.44	.41	.45	.66	.48	.35
C	.05	.33	.....	.50	.....	.48	.27
D	.05	.....	.....	.....	.42	.....	.42
Stems:							
A	.27	.06	.19	.25	.39	.12	.15
B	.27	.31	.20	.15	.18	.53	.19
C	.27	.06	.....	.52	.....	.26	.40
D	.27	.....	.....	.....	.39	.....	.21
Roots:							
A	.11	.21	.20	.22	.31	.26	.10
B	.11	.28	0.13	.13	.25	.25	.23
C	.11	0.21	.....	0.19	.....	0.19	.25
D	0.11	.....	.....	.....	0.31	.....	0.38

viously stored disappeared when the plants were changed from high to low moisture stress. Such large changes did not occur in content of inulin and free sugars; hence the levulins appear to be the important reserve carbohydrates.

TRAUB and SLATTERY (16) have pointed out that the levulins may be fractionated into two major portions, the 89%-ethanol-soluble and the 89%-ethanol-insoluble fractions. Their evidence also indicated that the former were of a much lower molecular weight and hence of lower polymer type than the latter. They also emphasized the importance of determining these two fractions in physiological experiments with guayule. In table 3 are shown the percentages, in the stems and roots of the plants, of the total levulins that are insoluble in 89% ethanol—the fraction with higher molecular weight. With one exception, plants grown under high moisture stress showed a greater proportion of their levulins to be of the higher-molecular-weight type than plants grown under low moisture stress. This one exception (treatment C, May, 1945, harvest) occurred while large quantities of levulins were accumulating (fig. 3*C*). In this instance the levulins may have been forming at such a rapid rate that the low-molecular-weight type was being produced at a greater rate than the high-molecular-weight type. When the moisture stress was changed from low to high, the percentage of the 89%-ethanol-soluble fraction of the levulins decreased; when changed from high to low, it increased. It is interesting that, although periods of high stress induced an increase in the proportion of high-molecular-weight levulins, it had little effect on the inulin content.

Under the conditions of the experiment there was no correlation between the moisture stress under which the plants were growing and the percentage of pentosans (table 4). The percentages increased to a maximum about the November 6 or the January 6 harvest and

then decreased to the end of the experiment. Thus, in contrast to the so-called "water-soluble carbohydrates" (table 2), the pentosan content was more affected by season of the year than by moisture stress.

RESIN CONTENT

The resin percentage was not affected greatly or in any consistent manner by moisture stress but did vary seasonally (table 5). It generally increased in leaves and stems up to the September and November harvests, respectively, and then decreased until the May 6 harvest. In the roots the percentage gradually decreased from the start to the close of the experiment.

The grams of resins per plant (stems and roots combined) on the various harvest dates increased steadily up to the

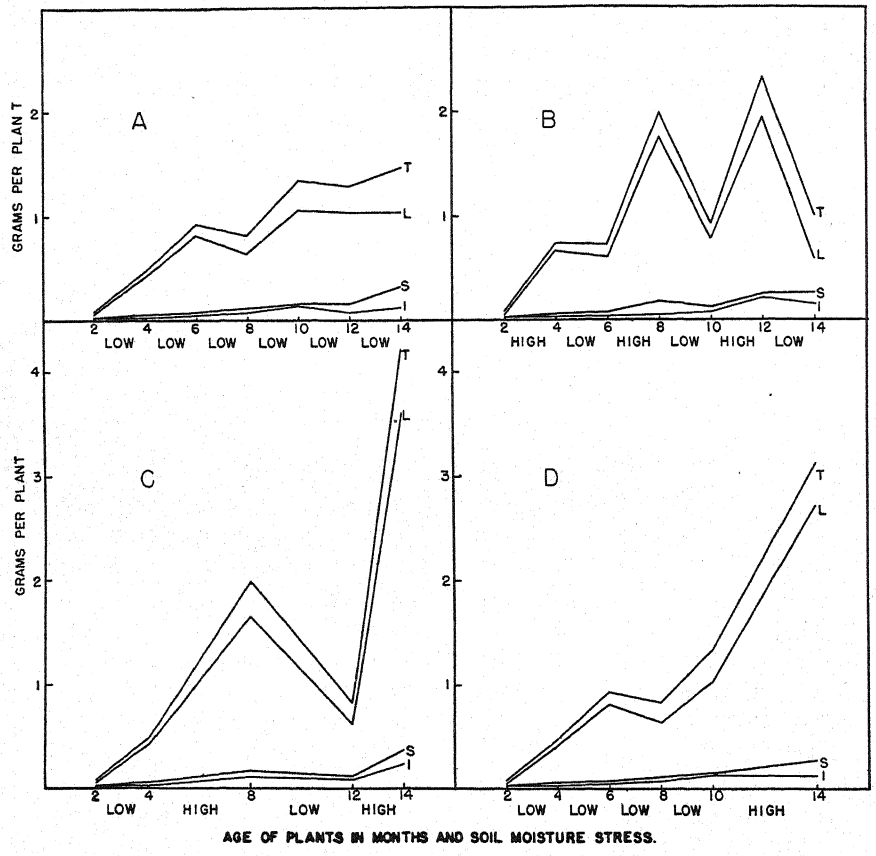


FIG. 3.—Grams of free sugars (*S*), levulins (*L*), inulin (*I*), and total (*T*) per plant in different treatments at indicated ages following periods of low or high soil moisture stresses.

Of the carbohydrates determined, the levulins were the most important reserve compounds. TRAUB (14) has shown that the pentosans may serve as reserve compounds under severe conditions, but it would seem that the conditions in these experiments did not induce their utilization in this manner.

November 6 harvest (fig. 4). Between November 6 and January 6, plants of treatments A, B, and D actually lost resins, but after January 6 they continued to gain in resins until the final harvest. The loss cannot be accounted for, but, because it occurred in all treatments, it is suggested that the season of the year from November 6 to January 6 is unfavorable for the formation of resins in guayule plants grown under these conditions of temperature and moisture in the greenhouse.

TABLE 3

PERCENTAGE OF LEVULINS INSOLUBLE IN 89% ETHANOL IN PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS*					
	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Stems:						
A	53.7	42.9	45.6	25.0	33.3	19.1
B	*65.0*	30.9	*58.1*	40.0	*52.4*	19.1
C	53.7	.....	*60.9*	.....	29.4	*34.4*
D	53.7	42.9	45.6	25.0	.....	*34.9*
Roots:						
A	38.4	37.2	34.7	20.4	35.9	30.8
B	*62.3*	33.9	*46.4*	30.9	*50.3*	41.0
C	38.4	.....	*53.1*	.....	34.3	*18.2*
D	38.4	37.2	34.7	20.4	.....	*47.8*

* Difference required at $P < 0.05$: between means, 10.1%; for interaction, 14.3%.

RUBBER CONTENT

The rubber content in the leaves was always less than 1% (table 6). The only significant change that occurred in the leaves in all four treatments was the marked increase in both percentage and grams per plant between the last two harvest dates.

During the first 8 months of the experiment or up to the November 6 harvest, the percentage of rubber in the roots and stems of plants growing under high moisture stress increased more, or decreased less, than the percentage in plants under low moisture stress. The grams of rubber per plant during this time increased more in the plants under high moisture stress than in those under low moisture stress (fig. 4).

From the November 6 harvest until and including the March 6 harvest, the percentage of rubber in the roots and stems in all treatments decreased, regardless of moisture stress. This decrease in percentage was greater in plants grown with low moisture stress following high moisture stress (treatment C) than in plants under continuous low moisture stress (treatment A). During this same period there was only a slight increase in grams of rubber in the roots and stems of plants grown under either high or low moisture stress.

Except for stems in treatment B, the percentage of rubber in the roots and stems of all treatments increased markedly from March 6, 1945, to May 6, 1945. The greatest increase was in plants of

TABLE 4

PENTOSAN CONTENT OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF PLANTS*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Per cent						
Leaves:							
A	2.13	2.72	3.33	2.79	2.13	1.58	1.69
B	2.13	*3.20*	3.13	*3.09*	2.00	*1.88*	1.97
C	2.13	2.72	.....	*3.27*	.....	1.72	*1.95*
D	2.13	.....	.....	.....	2.13	.....	*2.04*
Stems:							
A	2.13	2.46	2.86	2.54	2.42	2.37	1.97
B	2.13	*2.42*	2.64	*2.72*	2.17	*2.19*	1.82
C	2.13	2.46	.....	*2.68*	.....	2.45	*1.77*
D	2.13	.....	.....	.....	2.45	.....	*2.07*
Roots:							
A	1.35	1.43	1.37	1.19	1.49	1.26	1.06
B	1.35	*1.32*	1.39	*1.32*	1.53	*1.23*	1.10
C	1.35	1.43	.....	*1.36*	.....	1.31	*1.10*
D	1.35	.....	.....	.....	1.49	.....	*1.22*
	Grams per plant						
Leaves:							
A	0.049	0.264	0.712	0.815	0.713	0.686	1.187
B	.049	*.189*	.576	*.581*	.501	*.442*	1.077
C	.049	.264	.....	*.503*	.....	.576	*0.535*
D	.049	.....	.....	.....	.713	.....	*0.553*
Stems:							
A	.028	.105	.493	.596	.705	.874	1.254
B	.028	*.081*	.378	*.642*	.576	*.767*	0.998
C	.028	.105	.....	*.537*	.....	.694	*0.884*
D	.028	.....	.....	.....	.705	.....	*0.847*
Roots:							
A	.017	.058	.104	.108	.186	.167	0.193
B	.017	*.048*	0.077	*.116*	.162	*.136*	0.191
C	.017	0.058	.....	*0.114*	.....	0.147	*0.154*
D	0.017	.....	.....	.....	0.186	.....	*0.144*

* Difference required for significance ($P < 0.05$): between means of percentages, 0.35; between means of grams per plant, 0.206 for leaves, 0.183 for stems, and 0.35 for roots from November 6 harvest to end of experiment.

treatment C, which were growing under high moisture stress following a period of low moisture stress. The least increase—or a decrease—occurred in the plants of treatment B that were growing under low moisture stress following high moisture stress. The percentage of rubber in treatment D increased from January 6 to May 6. The grams of rubber per plant in the roots and stems increased markedly in all treatments in the last 2 months of the experiment. The increase was greatest in treatment C. An analysis of variance showed a highly significant interaction between the contents on March 6 and May 6 between treatments A and C. This indicated that the rate of accumulation of rubber was higher in treatment C than in treatment A.

The grams of rubber per plant in roots and stems are shown in figure 4 for the different treatments (*RU*), and, in addition, the grams of rubber in the plants grown under continuous low moisture stress (*RU-L*) are shown in sections *B*, *C*, and *D*. The progressively larger amounts of rubber in the plants at the end of each period of high moisture stress above that of the plants grown under continuous low moisture stress (treatment B, March harvest, excepted) are clearly shown. The highest yield obtained was from treatment C. Its final yield was 2.247 grams per plant as compared with 1.413 grams per plant in treatment A, a difference of about 59%. At the end of 8 months, treatment C had also yielded the most rubber per plant. At the close of the experiment, treatment D had produced barely significantly more rubber than treatment A. During the early part of the experiment, treatment B showed more rubber per plant at the end of periods of high moisture stress

TABLE 5

RESIN CONTENT (PERCENTAGE) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF PLANTS*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	3.42	5.72	6.63	4.40	3.31	4.43	4.99
B	3.42	*5.48*	5.61	*4.64*	3.53	*3.15*	5.79
C	3.42	5.72	.....	*5.38*	.....	3.87	*4.95*
D	3.42	.....	.....	.....	3.31	.....	*4.72*
Stems:							
A	4.27	5.13	4.70	6.34	4.54	4.46	4.39
B	4.27	*5.77*	5.13	*6.27*	3.94	*3.43*	3.09
C	4.27	5.13	.....	*6.89*	.....	4.57	*4.30*
D	4.27	.....	.....	.....	4.54	.....	*4.84*
Roots:							
A	6.05	5.69	4.03	5.50	3.13	3.17	2.65
B	6.05	*6.02*	4.13	*5.44*	3.80	*3.57*	2.86
C	6.05	5.69	.....	*5.64*	.....	4.04	*3.81*
D	6.05	.....	.....	.....	3.13	.....	*3.69*

* Difference required between means at $P < 0.05$ is 0.48%.

than treatment A, but at the end of the experiment there was no difference between them.

Discussion

The yield of rubber per plant is the product of the growth of the plant (dry weight) and of the proportion (percentage) of this weight that is made up of rubber. The results obtained in this experiment demonstrate two interacting conditions that affect these two determinants of rubber yield. These are moisture stress and some condition other than temperature associated with the season of the year, such as length of day, light intensity, or hours of sunlight. High moisture stress tended to retard growth, although the amount of retardation varied with the season. Thus, regardless of moisture stress, the plants in all treatments showed the highest rate of growth between March and May, 1945, and the lowest rate during the months just preceding (fig. 2). High moisture stress, except for the period from November 6 to March 6, tended to cause an increase in the proportion of rubber. This increase more than offset the decrease in growth rate, so that more

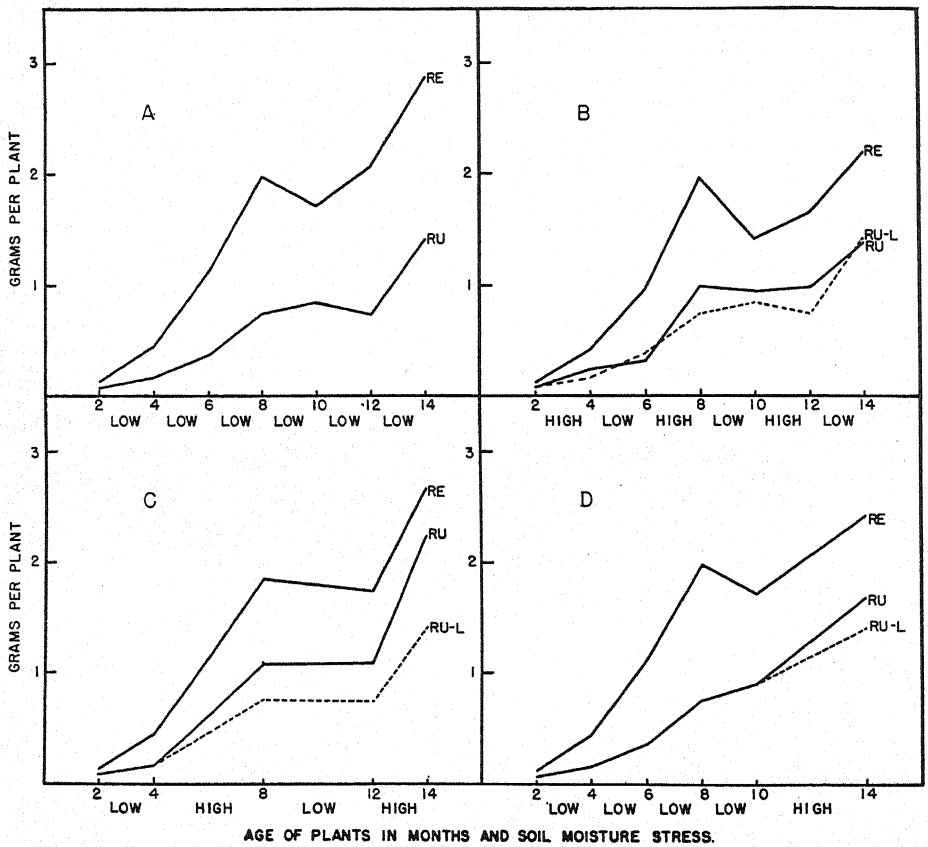


FIG. 4.—Grams of resin (*RE*); rubber (*RU*); and rubber in treatment A (*RU-L*), in roots and stems of plants in different treatments following growth under conditions of low and high soil moisture stress.

grams of rubber per plant were obtained under conditions of high moisture stress than low moisture stress.

The results show that guayule grown under alternating conditions of low and high moisture stress will contain more grams of rubber per plant than will plants grown continuously with low moisture stress and that the amount of the difference will be enhanced with each successive period of high stress. If the duration of these periods is too short, the increase in proportion of rubber will little more than offset the limitation on dry-weight increase so that the product of dry weight by rubber percentage will only slightly exceed that of plants grown in continuous low moisture stress. It is conceivable, also, that if the periods of high stress were too long, the growth of the plant might be so limited that the increased rubber content would not compensate the relative loss in dry weight in affecting the yield of rubber per plant. Under the conditions of these experiments, a 2-month high-stress period was too short and a 4-month high-stress period resulted in a much higher rubber yield under as nearly identical conditions as could be obtained (fig. 4, at age of 8 months). Under other conditions, or with older plants, stress periods of different duration would probably be more desirable. The ideal for which to strive would be a high-stress duration which would induce the maximum increase in proportion of rubber with minimum effect on the increase of dry weight.

If periods of high moisture stress are to be used practically in increasing the yield of rubber, the results emphasize the importance of the time of year in applying the high-stress treatments. During the period from November 6 to

TABLE 6

RUBBER CONTENT (PERCENTAGE) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

Plant part and treatment	Date of harvest and age of plants*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	0.31	0.35	0.39	0.42	0.34	0.18	0.64
B	0.31	*0.38*	0.52	*0.43*	0.30	*0.35*	0.47
C	0.31	0.35	.....	*0.44*	.....	0.29	*0.85*
D	0.31	.....	.....	.....	0.34	.....	*0.86*
Stems:							
A	2.52	0.41	1.25	2.16	1.84	1.50	1.70
B	2.52	*1.48*	1.18	*2.79*	2.25	*2.15*	1.79
C	2.52	0.41	.....	*3.56*	.....	2.83	*3.37*
D	2.52	.....	.....	.....	1.84	.....	*2.74*
Roots:							
A	3.32	3.64	2.02	2.65	2.50	1.40	1.82
B	3.32	*5.23*	2.69	*3.82*	3.35	*2.16*	2.37
C	3.32	3.64	.....	*4.48*	.....	2.58	*4.02*
D	3.32	.....	.....	.....	2.50	.....	*3.51*

* Difference required for significance ($P < 0.05$) between means: 0.13 for leaves, 0.65 for stems and roots.

March 6, conditions in this experiment were unfavorable to the formation of rubber, and periods of high stress did not induce an increase in rubber content. The most favorable time appears to be the spring, when conditions favored both growth and the accumulation of rubber. Plants subjected to high moisture stress during this period practically doubled their absolute rubber content in 2 months (treatment C [fig. 4]).

Because of the seasonal effect, unknown before the experiment was started, the results offer little information as to whether rubber accumulates in a full-grown plant or must be formed as the plant develops. They seem to indicate that, for highest rubber yield, the rubber should be accumulated in the plants as they grow, since the plants of treatment C had a higher rubber percentage and a greater absolute rubber content than those of treatment D. However, the plants of treatment D began and underwent the first half of their dry period during the season when no measurable amount of rubber was formed in any of the treatments. It may be that the rubber percentages in the plants in those two treatments would have differed less if their final dry periods had started and ended at the same time. The comparison of grams of rubber per plant in treatments B and C was made after 8 months instead of at the end of the experiment, because treatment B ended after a low-stress period and treatment C after a high-stress period.

Milled rubber from guayule contains as high as 20% of resin as an impurity. Any treatment which would reduce the resin content of the plant and increase its rubber hydrocarbon content would be highly desirable. However, in these experiments the grams per plant and percentage of resin varied in much the same manner as the rubber hydrocarbon in response to the various treatments.

Although season of the year influenced the amount of change in rubber and resin content of the plants brought about by conditions of high and low moisture stress, it had little effect on the changes in the carbohydrate content in response to these conditions. Thus the free-sugar, inulin, and pentosan content showed no marked response to changes in soil moisture stress at any season of the year. On the other hand, the levulin content always increased during periods of high moisture stress and decreased during the following periods of low moisture stress. This again points to the importance of the levulins as reserve carbohydrates.

Summary

1. Guayule nursery-transplants were grown for 14 months in alternating periods of low and high moisture stress of different durations. The dry weights and the carbohydrate, resin, and rubber contents of the plants were determined at the end of each period of moisture stress. During periods of high moisture stress, the rate of increase in dry weight of the entire plant was depressed, but most of this could be accounted for by the effect on leaf weight. The percentages of levulins, in roots and stems especially, during these periods increased markedly, while the percentages of the other carbohydrates—sugars, inulin, and pentosans—remained fairly constant. Except for the period from November 6 to March 6, the percentages of rubber increased during periods of high stress at a more rapid rate than during periods of low stress.

2. During periods of low stress following high stress, the percentage and absolute content of levulins per plant decreased. This indicated that the levulins

were serving as the principal reserve compounds, inasmuch as the amount of the other carbohydrates did not change greatly.

3. The rubber content on a grams-per-plant basis generally increased throughout the experiment, except for the period November 6–March 6, when it did not change. With this exception, the increase was greater during periods of high moisture stress than during low moisture stress. The absolute rubber content per plant was further increased, above that of plants continuously on low moisture stress, after each successive period of high moisture stress.

4. The results indicate that rubber accumulation can be forced in guayule plants by alternating periods of low and high moisture stress but that the periods of high stress must not be too short and, to induce maximum yields, should occur during seasons of the year other than the winter months.

LITERATURE CITED

1. ARTSCHWAGER, E. Growth studies on guayule. U.S. Dept. Agr. Tech. Bull. 885. 1945.
2. BONNER, J. Effects of temperature on rubber accumulation by the guayule plant. BOT. GAZ. 105:233–243. 1943.
3. BOUYOUCOS, G. J., and MICK, A. H. An electrical resistance method for the continuous measurement of soil moisture under field conditions. Mich. Agr. Exp. Sta. Tech. Bull. 172. 1940.
4. COOIL, B. J. Studies on the mineral nutrition of guayule. Report, Special Guayule Research Project, U.S. Dept. Agr. 1945.
5. HAISE, H. R., and KELLEY, O. J. The relation of electrical and heat conductivity to moisture tension in plaster of Paris blocks. Soil Sci. 61:411–422. 1946.
6. HENDRICKSON, A. H., and VIEHMEYER, F. J. Moisture distribution in soil in containers. Plant Physiol. 16:821–826. 1941.
7. HUNTER, A. S., and KELLEY, O. J. The growth and rubber content of guayule as affected by variation in soil moisture stress. Jour. Amer. Soc. Agron. 38:118–134. 1946.
8. KELLEY, O. J.; HUNTER, A. S.; HAISE, H. R.; and HOBBS, C. H. A comparison of methods of measuring soil moisture under field conditions. Jour. Amer. Soc. Agron. 38:759–784. 1946.
9. KELLEY, O. J.; HUNTER, A. S.; and HOBBS, C. H. The effect of moisture stress on nursery-grown guayule with respect to the amount and type of growth, and growth response on transplanting. Jour. Amer. Soc. Agron. 37:194–216. 1945.
10. LLOYD, F. E. Guayule (*Parthenium argentatum* Gray)—a rubber-bearing plant of the Chihuahuan Desert. Carnegie Inst. Wash. Publ. 139. 1911.
11. MCRARY, W. L., and SLATTERY, M. C. The colorimetric determination of pentoses and pentosans. Arch. Biochem. 6:151–156. 1945.
12. TIPPETS, L. H. C. Tracts for computors, random sampling numbers. Cambridge University Press, London. 1927.
13. TRAUB, H. P. Rapid photometric methods for the determination of rubber and resins in guayule tissue, and rubber in crude rubber products. U.S. Dept. Agr. Tech. Bull. 920. 1946.
14. ———. Concerning the function of rubber hydrocarbon (caoutchouc) in the guayule plant, *Parthenium argentatum* A. Gray. Plant Physiol. 21:425–444. 1946.
15. TRAUB, H. P., and SLATTERY, M. C. Analysis of levulins, inulin, and monosaccharides in guayule. BOT. GAZ. 108:295–299. 1946.
16. ———, and ———. Levulins and inulin in guayule (*Parthenium argentatum* A. Gray). Plant. Physiol. 22:77–87. 1947.

EFFECTS OF FRUIT THINNING ON ALMOND FRUITS AND SEEDS

GEORGE A. BAKER[1] AND REID M. BROOKS[2]

Introduction

In 1939 BROOKS (1) initiated studies on growth of the fruit and seed of the almond; growth was presented as averages of measurements at uniform time intervals on the linear dimensions of various parts of fruits and seeds of the Nonpareil and Texas varieties. The present study will indicate the effects of thinning for Nonpareil on the averages and variances (as functions of time) of a linear-dimension, fruit-cheek diameter; on the means, variances, and correlations for three variables, namely, final fruit-cheek diameter, seed-cheek diameter, and seed weight; on the yield of seed per 100 leaves; and on the weight of new terminal wood per 100 leaves.

Material and methods

A mature, healthy, bearing tree of Nonpareil was selected in the almond-variety collection of the University of California at Davis; a fundamental assumption (3) was made that each limb (2 inches in diameter or larger) behaves as a unit independent of adjacent limbs. On March 21, 1944, on this tree, 335 fruits were tagged and their cheek diameters measured. They were selected by inspection as the largest per spur (average cheek diameter of 6.942 mm.) and were well scattered among the lower branches of the tree; these almonds are referred to as "unthinned." On the same day a branch about 2 inches in diameter on the southeast side was thinned to the one largest fruit per spur, all removed fruits being saved and measured. The total, including those left on the branch, was 2766 fruits, with an average cheek diameter of 4.984 mm. and with a standard deviation of 1.119 mm. The lot of 350 tagged fruits left on the branch is called the "first thinning" and had an average cheek diameter of 6.908 mm.

On March 22, 1944, another branch, about $4\frac{1}{2}$ inches in diameter, on the opposite side of the tree was selected and thinned more severely, the largest fruit per spur again being selected with each selected fruit at least 6 inches from its closest neighbor. The removed fruits were saved and measured. In all there were 1623 fruits (including those left on the branch) with an average cheek diameter of 5.360 mm. and with a standard deviation of 1.110 mm. The lot of 188 tagged fruits remaining on the limb is known as the "second thinning" and had an average cheek diameter of 7.149 mm.

On May 16 and 23, 1944, all fruits remaining on a $1\frac{1}{2}$-inch limb near the first thinned limb were tagged and measured. Some of these 65 fruits had been measured throughout the season. This lot is the "unthinned $1\frac{1}{2}$-inch branch."

This variety was in full bloom on March 4, 1944. At the time of thinning the jackets (floral envelopes) had split on the majority of fruits. None of the jackets had fallen, but all were removed before measurements were made.

One can understand the difference in thinning more precisely by observing that on May 11 the number of leaves per original tagged fruit was eighteen for the first thinning and fifty-eight for the second thinning. The cheek diameters of these fruits were measured again on

[1] Assistant Statistician, [2] Assistant Pomologist; California Agricultural Experiment Station, Davis.

March 28; April 4, 11, 18, 25; and May 2, 9, 16, 23. On August 8 the fruits were mature and were harvested; weights of seeds on a comparable moisture basis were recorded on August 17–19. The weight of wet new terminal wood on each branch included in the experiment was determined at harvest time by clipping and weighing the current-season growth in length.

The standard deviation of an individual measurement on cheek diameter of a fruit was estimated from the last two sets of measurements to be 0.23 mm.

Results and discussion

Effect of thinning on growth and dispersion of fruit-cheek diameter

It is to be expected that thinning will result in increased relative size of fruits.

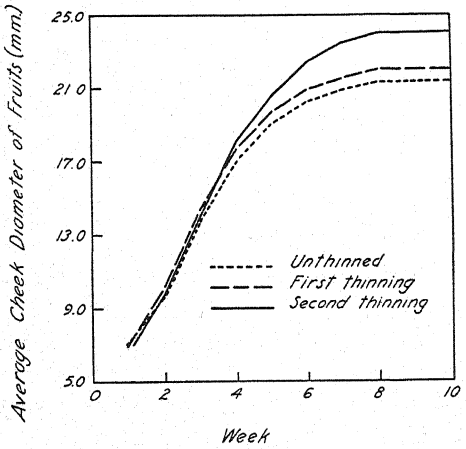


Fig. 1.—Average cheek diameters at weekly intervals for Nonpareil almonds for three degrees of thinning, Davis, California, 1944.

Figure 1 shows the extent to which this rule held true for fruits that remained on the tree until harvest. From the values of averages and variances (figs. 1 and 2) it follows that the averages of the fruit-cheek diameters of unthinned and first thinning did not differ significantly at the beginning of the period (March 21, 1944). The average for the second thinning was slightly higher than for the other two categories; but the measurements were made the next day, March 22—a fact that probably accounts for the difference. At the end of the season the

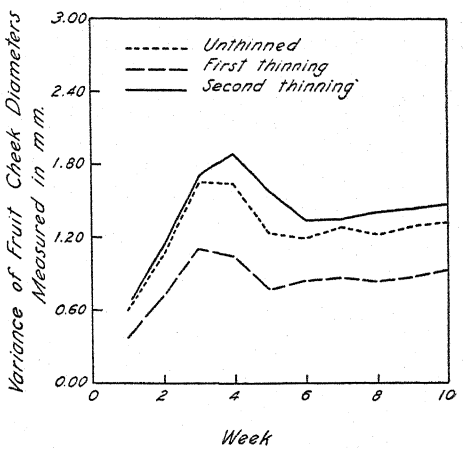


Fig. 2.—Variances at weekly intervals of the cheek diameters of Nonpareil almonds for three degrees of thinning, Davis, California, 1944.

averages of the fruit-cheek diameters of all categories were statistically significantly different.

The effect of thinning on the variances of the cheek diameters of fruits that remained on the tree until harvest seems to be less consistent. The variance curves (fig. 2) for the different categories are strikingly parallel throughout the season, with distinct peaks in midseason. In all cases the variances increase, reach a maximum value, decrease, reach a relative minimum value, and then increase slightly. These features of the variance curves probably reflect the nonsynchronism of the growth curves of individual almond fruits.

At the beginning of the season the variances for both the unthinned and the second thinning were greater than the variance of the first thinning. At the

end of the season the variance for the second thinning is significantly greater than for the first thinning ($P < 0.01$); the variances for the unthinned and second thinning are not significantly different; the variances for unthinned and first thinned are significantly different ($P < 0.05$). These differences in variances do not result from differences in methods of selection, since the largest fruit per spur was selected in each case. In general, one would expect the unthinned fruits to show as great variation as any of the other categories, or greater, since these fruits are a sample from a population subject to a wider range of conditions. The first-thinning fruits were very uniform as compared with the others. Once the selection was made, the relative positions of the categories with respect to size of variance did not change throughout the season. Thinning seems not to be effective in changing the relative variability of the cheek diameter of fruits in the several categories. Apparently, however, its effect on seeds is consistent and marked.

TABLE 1

NUMBERS OF MEASURABLE SEEDS, AVERAGE SEED WEIGHTS, AVERAGE SEED-CHEEK DIAMETERS, AND VARIANCES FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	No. of seeds	Average seed weight (gm.)	Variance of seed weight	Average seed cheek (mm.)	Variance of seed cheek
Unthinned 1½-inch branch	37	1.0710	0.00835	8.03	0.3909
Unthinned	115	1.0552	.01582	7.84	.3509
First thinning	250	1.1331	.01089	7.77	.2453
Second thinning	153	1.2495	0.00971	7.48	0.2075

MEANS, VARIANCES, AND CORRELATIONS FOR SEED WEIGHT, SEED-CHEEK DIAMETER, AND FINAL FRUIT-CHEEK DIAMETER

The average weights of seeds for the unthinned, first thinning, and second thinning are highly significantly different and are in the order named for increasing weight (table 1). The variance of the unthinned seed weights is greater than those of the first thinning ($P < 0.05$) and second thinning ($P < 0.01$). The variance of the first-thinning seed weights is not significantly different from that of the second thinning, but the variances do decrease as the degree of thinning increases.

The unthinned do not differ significantly from the first thinning in average seed-cheek diameter. The first thinning and second thinning, also the unthinned and second thinning, are highly significantly different in this measurement. The order for decreasing seed-cheek diameter is unthinned, first thinning, and second thinning. The variance of seed-cheek diameter for unthinned is greater than the variances for the first thinning ($P < 0.05$) and second thinning ($P < 0.01$), but these variances for the first thinning and second thinning do not differ significantly. There is, however, a steady progression downward in the variance of seed-cheek diameters as the degree of thinning increases.

Thinning does not seem to have any striking or consistent effect on correlations between pairs of the following variables: final fruit-cheek diameter, seed-cheek diameter, and cube root of

weight (table 2). The cube root of weight is used in order that the regression equations involving weight may be dimensionally correct. The correlations between final fruit-cheek diameter and cube root of weight of the seed are uniformly high. The correlations between seed-cheek diameter and cube root of weight of seed run fairly uniformly low. Since in the latter two cases a rough approximate standard error of a difference is 0.12, few (if any) of the differences appear significant. A similar standard error for differences in correlation coefficients for final fruit-cheek diameter with cube root of seed weight is 0.05; again, the differences between categories do not appear to be especially significant. Perhaps the coefficient is low for the unthinned. For more exact testing see DAVID (2).

TABLE 2

CORRELATIONS FOR SEED-CHEEK DIAMETER, FRUIT-CHEEK DIAMETER, AND CUBE ROOT OF SEED WEIGHT FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	No. of seeds	Seed cheek vs. cube root of weight	Seed cheek vs. fruit cheek	Fruit cheek vs. cube root of weight
Unthinned 1½-inch branch..	37	0.27	0.29	0.83
Unthinned.....	115	.57	.26	.63
First thinning.....	250	.45	.48	.82
Second thinning.....	153	0.39	0.23	0.72

In summary, thinning causes a relative increase in weight of seeds and a decrease in variability of the weights. The average seed-cheek diameters are less with increase of degree of thinning. Since, however, the weight increases with degree of thinning, evidently the shape of the almond has changed, becoming longer, broader, or both, but smaller in cheek diameter. These facts partially explain the low correlations between seed-cheek diameter and cube root of seed weight and between seed-cheek diameter and final fruit-cheek diameter. Wrinkling of the seed coats and of the outer portion of the cotyledons may also be a factor. The correlations between final fruit-cheek diameter and cube root of weight of seed run much higher.

That thinning induces a change in shape of almond seeds is borne out by certain observations on peaches. DAVIS (3) noticed that peaches thinned very early had much larger pits; that the seeds were long, with large suture diameters but small cheek diameters; and that the seed coats were wrinkled as compared with those in peaches thinned at a later date. Germination of early-thinned seeds was very low compared with later-thinned ones. These facts raise the question as to how the seed material is stored and in what order. Evidently it is laid down in the middle section of the seed in the plane of the suture and length diameters and gradually fills out as much as possible in the cheek diameter. Thus in early thinning the pits of peaches or the outside (pericarp) dimensions of almonds increase very rapidly early in the season because of the decrease in competition with fruits that normally would stay on relatively late. By the time seed material begins to be stored, the space available for seed growth is large; the lack of leaf surface and of time, as well as competition with wood growth, prevents this space from being completely filled. Al-

though the resulting seeds are heavier, they are thinner in cheek diameter and have wrinkled seed coats.

The unthinned 1½-inch branch almonds did not differ significantly from the unthinned in average weight of seed, average seed-cheek diameter, or variance of seed-cheek diameters. The probability, however, is between 0.01 and 0.05 that the unthinned are more variable in seed weight than the unthinned 1½-inch branch ones. It is important that these two samples can be regarded as random samples from populations with the same mean, since some of the later analysis required information for the unthinned category, which is available only for the 1½-inch branch.

It should be borne in mind in these tests for significant differences that the fruits and seeds in each category may differ from the fruits and seeds in the other categories in both their means and variances.

Effect of thinning on yield of seed

As we have seen, thinning affects the weight of seed. It tends to increase the size of individual seeds; but with increase of thinning there are fewer seeds. We wish to express as accurately as possible the relation between thinning and yield. As a measure of thinning we shall take the number of leaves per harvested fruit (x) and shall express yield in terms of grams of seed per 100 leaves (y). If we plot the logarithms to the base 10 of y (Y) against the logarithm to the base 10 of x (X), we obtain three points that very strikingly lie on a straight line. The linear trend, even though based upon only three points, is statistically very highly significant ($P = 0.0005$). Table 3 gives the details of determining the relation between X and Y.

TABLE 3

LEAVES PER HARVESTED FRUIT (x) AND YIELD OF SEED IN GRAMS PER 100 LEAVES (y) FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	x	$X = \log x$	y	$Y = \log y$	Calculated Y
Unthinned 1½-inch branch	17	1.23045	6.399	0.80611	0.80594
First thinning	25	1.39794	4.506	.65379	.65401
Second thinning	70	1.84510	1.772	0.24846	0.24839

$Y = -0.90710X + 1.92208.$

$F = \frac{0.1662}{0.0000000822} = 2{,}022{,}000$ with 2 and 1 degrees of freedom.

$P = 0.0005.$

The corresponding relation between the variables x and y is

$$y = \frac{83.58}{x^{0.90710}}. \qquad (1)$$

If the weights of the individual seeds do not change with degree of thinning, then y would vary inversely as x—that is, $y = k/x$. But since the weight of the individual seeds increases with the degree of thinning, y decreases more slowly than is indicated by $y = k/x$. Thus the difference 1.00000 − 0.90710 measures the ability of the remaining fruits to utilize the increasing leaf surface per fruit. Part of the energy made available by the leaves of a limb goes into the production of new wood as thinning becomes more extreme.

Effect of thinning on growth of new wood

Thinning may be expected to promote the growth of new wood. If we designate the number of leaves per harvested fruit by x (as before) and the grams of new terminal wood per 100 leaves as observed August 10 by w, then we find that

$$w = 0.029333x - 0.47552 . \quad (2)$$

The details of fitting appear in table 4. The trend is again statistically significant ($P = 0.02$), even though we are dealing with only three points. From equation (2) w is zero when $x = 16.2$. Thus, roughly, the weight of new terminal wood per 100 leaves is proportional to the number of leaves per harvested fruit minus the number of leaves per harvested fruit for which there is no new terminal wood growth.

TABLE 4

Leaves per harvested fruit (x) and weight in grams of new wood per 100 leaves (w) for Nonpareil almonds for different degrees of thinning, Davis, 1944

Degree of thinning	x	w	Calculated w
Unthinned 1½-inch branch	17	0.0000	0.0231
First thinning	25	0.2850	0.2578
Second thinning	70	1.5737	1.5778

$w = 0.029333x - 0.47552.$

$F = \frac{1.4049}{0.001290} = 1089.$

$P = 0.02.$

Summary and conclusions

1. Thinning causes an increase in the average weights of the seeds of Nonpareil almond with a decrease in the variability of the weights. This is in contrast with the effect of thinning on variability in fruit-cheek diameter. The cheek diameter of seeds and its variability are less with increased degree of thinning. The result is a distinct change in shape of the almond seeds. That is, heavily thinned almonds are long and broad across the suture diameter and flat or narrow in the cheek diameter. The correlations of final fruit-cheek diameter with seed-cheek diameter and of seed-cheek diameter with the cube root of the seed weight are very low. The correlations of final fruit-cheek diameter with the cube root of seed weight are much higher.

2. When the logarithms of yield of seed in grams per 100 leaves (Y) are plotted against the logarithms of the number of leaves per harvested fruit (X), a very striking straight-line relation results, with the coefficient of X somewhat less than 1 (0.90710). If the number of leaves per fruit did not affect the size of the seeds, then the coefficient of X would be 1.

3. If the weight of new terminal wood in grams per 100 leaves is plotted against the number of leaves per fruit at harvest time, the three observed points lie almost exactly on a straight line. Thus, approximately, the weight of new terminal wood per 100 leaves is proportional to the number of leaves per fruit minus the number of leaves per fruit for which there is no new terminal wood.

4. The results of an investigation on the effect of thinning on the fruits and

seeds of Nonpareil almonds have been presented in detail. A parallel study on the Texas variety in the same season on a closely situated similar tree gave almost identical results. The latter test is not published in detail because it adds nothing new. It does, however, give valuable support to the Nonpareil experiment.

DIVISION OF MATHEMATICS AND PHYSICS
DIVISION OF POMOLOGY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

LITERATURE CITED

1. BROOKS, R. M. A growth study of the almond fruit. Proc. Amer. Soc. Hort. Sci. 37:193–197. 1939.
2. DAVID, F. N. Table of the correlation coefficient. Biometrika Office, University College, London. 1938.
3. DAVIS, L. D. Unpublished data. On file with Division of Pomology, University of California, Davis. 1935.

EFFECT OF DURATION AND INTENSITY OF LIGHT UPON FLOWERING IN SEVERAL VARIETIES AND HYBRIDS OF CASTOR BEAN

N. J. SCULLY[1] AND W. E. DOMINGO[2]

Introduction

In certain populations of castor bean a relatively small percentage of the plants fail to produce flowers, although all are subjected to the same field conditions. Such failure has been recently investigated on a genetical basis by DOMINGO (5), who grew plants throughout the entire growing season at Beltsville, Maryland, and found that the flowerless character was evident only in the segregating generations of certain crosses. In such crosses plants of both the parental and the F_1 populations flowered, and the percentage of flowerless individuals was relatively uniform for the F_2 populations investigated.

Since floral initiation and development in numerous plant species were known to be strikingly controlled by photoperiod, it seemed desirable to determine what effect this factor might have upon the flowering responses of parents, their F_1 progeny, and those F_2 individuals that exhibit the flowerless character.

At Beltsville the growing season for castor bean extends from early May until the middle of October. The length of day from sunrise to sunset increases from $13\frac{1}{2}$ hours on May 1 to $14\frac{9}{10}$ at the solstice, June 21, and then decreases to $11\frac{1}{4}$ hours on October 15. There is some additional twilight both night and morning that may be photoperiodically effective for castor bean, but to what extent is not known. It thus seemed possible, if a photoperiod factor is involved in the flowering of this species, that the critical photoperiod for the flowerless types might be either longer or shorter than natural durations available locally.

Since a flower-inducing stimulus has been transmitted from one variety of soybean to another by grafting, this procedure was also tested for castor beans.

The present study considers the ef-

[1] Associate Physiologist and [2] Agronomist, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Md.

fects of photoperiod and grafting upon the flowering of castor-bean plants.

Material and methods

Earlier work (5) had indicated that F_2 populations from crosses between the parent varieties Kentucky 38 and D.P. 227 contained some plants exhibiting the flowerless character. These parents and individuals of their F_1 and F_2 progenies comprised the bulk of the plant material in these experiments. In addition, several domestic varieties or selections were investigated in the initial photoperiodic experiment.

Plants were grown in soil in clay pots or soil beds in the greenhouse, in clay pots in controlled-environment rooms, and in soil beds or field plots in field experiments. Plants grown in the greenhouse received temperatures of about 75° F. and those in the controlled-environment rooms of about 70° F.

Only photoperiods of natural or shorter than natural duration were used in the field. To provide short photoperiods in the field experiments, frames covered with a double thickness of black sateen cloth were placed over the plants at the end of the light period. In the greenhouse, photoperiods of shorter duration than those occurring naturally were obtained by moving the plants into dark chambers at the close of the light period. For longer photoperiods, supplemental incandescent-filament light was used beginning at sunset, either in the greenhouse or in chambers adjacent to it. The intensities of supplemental light varied from 10 to 150 foot-candles.

Plants in the controlled-environment rooms (8) received all their light from a carbon-arc lamp supplemented with incandescent-filament lamps. The intensity of light in these rooms was approximately 2000 foot-candles at pot level.

The place of formation of first flower buds (number of nodes from base of plant) was determined for the various plants rather than the date of opening of the first flowers. This criterion has certain advantages, most important of which is that it is a more accurate measure of the time when flowers were initiated, since it furnishes earlier evidence of initiation and thus shortens the interval during which errors might arise owing to variation among plants in rate of development. It also permits the detection, by dissection, of initiated flowers in certain plants which are killed by frost before blossoming. Plants that were found not to have initiated flowers at the end of the experiment were classed as nonflowering, and the total number of nodes for each was recorded.

Experimentation and results

Experiment I

The purpose of this experiment was to test for any outstanding influence of length of photoperiod upon either the formation of flower primordia or the rate of production of nodes. Seed of nine varieties or hybrid lines of castor bean were planted on August 3, 1944, in 8-inch pots in the greenhouse. Only a limited amount of seed of some of these was available, and, as a result, varying numbers of individuals were present in the different populations. The varieties Doughty 11 and Kentucky 38 were grown in thirty-two pots, and sixteen pots of each were placed on each of two greenhouse trucks. Two other trucks each carried eight pots of the Conner variety and four pots of each of the other six varieties.

Beginning with seedling emergence, one lot containing plants of all varieties was subjected to 8-hour photoperiods of

natural daylight, while a comparable lot received approximately 18-hour photoperiods, the latter consisting of all the natural daylight available plus supplemental Mazda light of about 40 foot-candles intensity from sunset until 12:00 M.

Beginning September 1 and ending September 22, weekly dissections were made of several plants of Kentucky 38 and Doughty 11 varieties on both photoperiods to determine their stage of development. Only plants of Kentucky 38 on the longer photoperiod were found with flower primordia or buds on each of these dates. However, on September 9, varieties 3/12 and 3/31 on both photoperiods each had its first flower clusters macroscopic in size and emerging from the sheaths surrounding the inflorescences. This same stage of development was attained at this time by plants of Kentucky 38 on the long photoperiod. The remaining varieties did not then have macroscopic flower buds.

Since plants of the Kentucky 38 variety were flowering promptly on long but not on short photoperiod, a group of four from short photoperiod were transferred to long photoperiod on September 4. On September 16 all four of these had initiated first flowers above the thirteenth node, while corresponding plants remaining on short photoperiod had produced thirteen nodes and were vegetative.

On October 18 the experiment was terminated, and the node at which the first flower primordia occurred, or the total number of nodes in vegetative plants, was recorded for the remaining plants (table 1).

TABLE 1

EFFECT OF PHOTOPERIOD ON SEVERAL VARIETIES OF CASTOR BEAN IN GREENHOUSE EXPERIMENT I. PLANTED AUGUST 3, 1944. HARVESTED OCTOBER 18, 1944

VARIETY	8-HOUR PHOTOPERIOD				18-HOUR PHOTOPERIOD			
	Flowering plants		Vegetative plants		Flowering plants		Vegetative plants	
	No.	Average node at which first flower primordia occurred	No.	Total nodes produced per plant	No.	Average node at which first flower primordia occurred	No.	Total nodes produced per plant
Kentucky 38	0	.....	5	19.0	16	8.4	0	.....
Doughty 11	0	.....	8	19.0	0	.....	8	21.1
Conner	2	12.0	5	17.0	2	17.0	6	17.7
D.P. 58	0	.....	4	17.5	0	.....	4	17.5
D.P. 33	0	.....	4	17.0	0	.....	4	16.5
3/103	0	.....	4	17.5	0	.....	4	17.8
3/31	4	10.8	0	.....	4	11.3	0	.....
3/180	1	10.0	3	17.0	4	13.0	0	.....
3/12	4	8.5	0	.....	4	9.0	0	.....

The most striking result of the different treatments was the flowering response of the Kentucky 38 variety. All plants flowered on the longer photoperiod, but none did so on the shorter one. The average node position of first flower primordia was 8.4 in the former lot as determined at harvest time, while the plants on 8-hour photoperiod were vegetative at harvest and had produced

an average of nineteen nodes. Figure 1 shows one representative plant each from the 8- and the 18-hour photoperiod lots. The response to the longer daylength was obviously prompt, since plants of all varieties had an average of six nodes at the time of thinning when only their cotyledons were expanded.

Responses of the remaining eight varieties were variable. Doughty 11, D.P. 58, D.P. 33, and 3/103 failed to flower on either photoperiod, and the total nodes formed were approximately the same for each of these varieties regardless of treatment. Plants of two other varieties, 3/31 and 3/12, flowered on both photoperiods, and with each the average node position of first flowers was a fraction of a node higher on long than on short photoperiod. Some of the Conner plants flowered on each photoperiod, but the first flowers were at a substantially higher position on the longer one. All four of the 3/180 plants on the longer photoperiod flowered, while only one of four flowered on the shorter one.

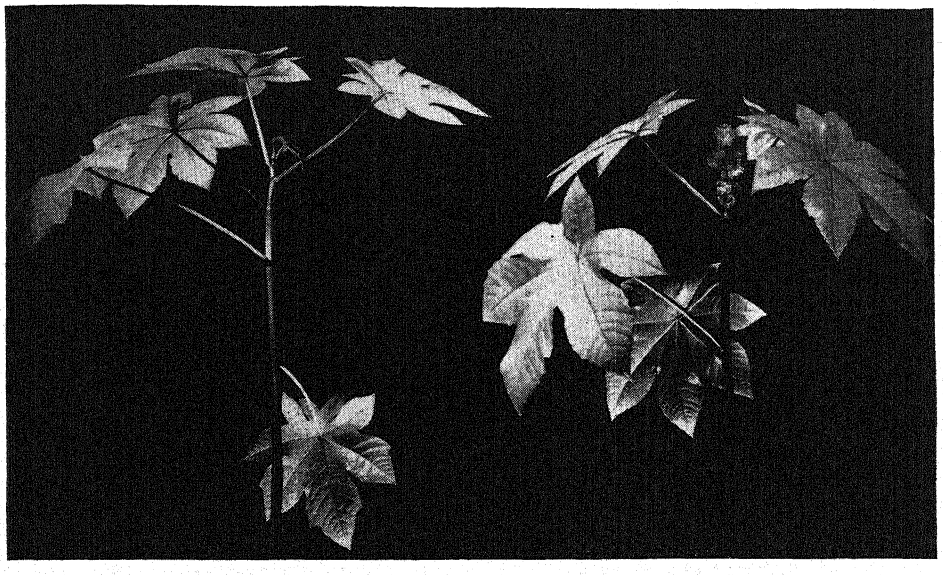


FIG. 1.—Castor-bean plants of Kentucky 38 variety grown with different photoperiods: *left*, 8-hour; *right*, approximately 18-hour. (Photographed September 27, 1944.)

EXPERIMENT II

This experiment was designed to determine more accurately the critical photoperiod of variety Kentucky 38. On September 4, 1944, a large planting of seed was made in the greenhouse in 6-inch pots. Prior to germination the latter were divided into six equal lots of sixteen pots each. Five lots were placed on photoperiods of 8, 10, 12, 14, and 16 hours, which consisted of 8 hours of natural daylight plus supplemental incandescent light of 40 foot-candles intensity. The sixth lot was subjected to all the natural daylight available plus supplemental light from about sundown until midnight in order to approximate the long photoperiod in experiment I. This photoperiod was $17\frac{1}{4}$ hours long at the start of experiment II.

On October 3, 1944, two plants from

each lot were dissected, and all were vegetative, with ten nodes each. The remaining plants were still vegetative when dissected on October 30 and averaged thirteen to fourteen nodes.

In flowering response to photoperiod, the results of this experiment were in contrast to those of experiment I.

EXPERIMENT III

This experiment was designed to find why opposite responses to long photoperiod were obtained with variety Kentucky 38 in experiments I and II. Experiment III*A* was concerned with various lengths of photoperiod consisting of natural light supplemented with different intensities of artificial light; experiment III*B*, with effects of long photoperiods with artificial light of high intensity in a plant-growth chamber; and experiment III*C*, with effects of long and short photoperiods on plants grown in midwinter, when total natural radiation was less than at other seasons of the year.

EXPERIMENT III*A*.—Seed was planted in the greenhouse in 6-inch pots on September 9, 1944. The population was divided into eight lots of twenty-two plants each. One lot each was placed on 8-, 10-, and 24-hour photoperiods. Each of these photoperiods comprised 8 hours of natural daylight, and in the case of the two longer ones the remaining light period consisted of incandescent light of 25 foot-candles intensity. Four other lots were placed on 16-hour photoperiods consisting of 10 hours of natural daylight plus 6 hours of incandescent light at intensities of 10, 25, 50, or 150 foot-candles. The eighth lot was subjected to a photoperiod comprising all natural daylight available plus incandescent light of 25 foot-candles intensity from sunset to 12:00 M. This photoperiod was 16 hours and 56 minutes long when the plants of the various lots were thinned on October 11, 1944.

On October 27, 1944, half of the plants receiving 24-hour photoperiod were dissected. All were vegetative, and each had nine nodes. On November 18, 1944, the remaining plants on 24-hour photoperiod were all found to be vegetative, with an average of thirteen nodes. The other treatments were continued until December 18, 1944, at which time all lots were still vegetative. Although the average number of nodes produced by the various lots were quite similar, certain of them differed by amounts which are statistically significant (table 2). Plants on 16-hour photoperiod with supplemental light of 150 foot-candles intensity produced significantly more nodes than those in four other lots receiving either lower intensity of supplemental light or a shorter photoperiod.

No Kentucky 38 plants flowered when subjected to long photoperiods during the fall and winter months in experiments II and III*A*. Accordingly, it seemed desirable to determine whether flowering would occur in this variety if maintained on long photoperiod of a continuous and relatively high intensity such as is available in the controlled-environment rooms at Beltsville (8).

EXPERIMENT III*B*.—On November 28, 1944, seed of Kentucky 38 was planted in 4-inch pots. The plants were kept on 8-hour natural photoperiod in the greenhouse until December 8, at which time the cotyledons were expanded on all but a few plants. Twenty-eight of them were then moved to a controlled-environment room at 70° F. and initially with 15-hour photoperiod. Since the room was being used for additional experiments, a longer photoperiod was not attainable. On December 17, 1944, the photoperiod was changed for other

purposes to 18 hours and again back to 15 hours on January 2, 1945.

On January 26 the plants were dissected and ten had initiated flower primordia at the average position of node 8.6. The other eighteen were vegetative and averaged 11.1 nodes per plant. The position of first flowers was only 0.2 node higher than for the same variety in experiment I. However, since only ten of twenty-eight plants flowered as against all plants flowering in the earlier experiment, a valid comparison could not be made.

The purpose of experiment III*C* was to observe whether plants of this variety would remain vegetative on relatively long photoperiods at this time of year. Such long photoperiods would contain a substantially shorter duration of natural daylight than would those employed during August to October in experiment I.

After growth until March 1, 1945, dissection showed that all plants were vegetative in all lots. Those which had been on 8-hour photoperiods averaged 10.9, 9.8, 8.8, and 8.0 nodes, respectively, for the four successive planting dates, while the series on long photoperiods averaged 11.2, 10.1, 9.1, and 8.0 nodes.

TABLE 2

EFFECT OF PHOTOPERIOD AND INTENSITY OF SUPPLEMENTAL LIGHT ON DEVELOPMENT OF KENTUCKY 38 VARIETY CASTOR BEAN IN GREENHOUSE. EXPERIMENT III*A*. PLANTED SEPTEMBER 29, 1944. HARVESTED DECEMBER 18, 1944

Photoperiod (hours)	Natural daylight (hours)	Supplemental light		No. of plants	No. of nodes per plant
		Duration (hours)	Intensity (foot-candles)		
8	8	0	.....	21	14.9±0.1*
10	8	2	25	21	15.5±0.1
16	10	6	150	20	16.0±0.1
16	10	6	50	20	15.7±0.2
16	10	6	25	22	14.9±0.1*
16	10	6	10	22	14.8±0.2*
16†	†	†	25	21	15.0±0.1*

* Significantly different from 16-hour, 150 foot-candles lot, at the 1% level.

† This photoperiod consisted of all natural daylight plus supplemental light from sunset to midnight.

EXPERIMENT IIIC.—Beginning December 27, 1944, four successive weekly plantings of Kentucky 38 seed were made in 4-inch pots. There were two lots of twenty plants each on each planting date, which were placed on short and long photoperiods. The short photoperiod consisted of 8 hours of natural daylight. The long one comprised all natural daylight plus Mazda light of 25 foot-candles intensity until 12:00 M. and varied from 16 hours at the start to 16½ hours at the end of the experiment.

EXPERIMENT IV

It was noted earlier that F_2 populations from crosses between varieties Kentucky 38 and D.P. 227 contained plants that failed to flower when grown in the field at Beltsville. DOMINGO (5) found that a large percentage of the plants formed their first flower primordia early in the season at a relatively low

node position but that a few formed them late in the fall, and therefore at a very high node position, and that about 10% remained vegetative throughout the entire growing season.

Experiment IV was mainly designed to determine what effects a longer growing season and varied photoperiod treatments would have upon the flowering responses of the parental varieties, Kentucky 38 and D.P. 227, and of their F_1 and F_2 progenies and to study in more detail the flowering behavior of Kentucky 38 plants grown with long photoperiods during late winter and spring months.

EXPERIMENT IV*A*—On February 1, 1945, seed of both parents, the F_1, and six F_2 populations was planted in 8-inch pots in the greenhouse. The nine different populations were grouped separately and, following germination, were subjected to a $14\frac{1}{2}$-hour photoperiod consisting of all natural daylight plus incandescent light applied both before and after the natural day. The supplemental light period was regularly shortened as the length of natural day increased. The $14\frac{1}{2}$-hour photoperiod was selected, since it closely approximated the natural photoperiod to which castor beans were subjected following emergence in the field in May. Supplementary light was used in the greenhouse until May 6, when the natural daylength was longer than $14\frac{1}{2}$ hours.

In the various populations the plants that produced visible flower buds were harvested, and the position of first flowers recorded. By May 23 all plants of the parental and F_1 populations as well as 436 of the original 546 F_2 plants had produced flowers.

The 110 F_2 plants that had not produced visible flowers by May 23 were divided into four lots. Three of the lots contained 18 plants each, including individuals from all six F_2 populations, and received photoperiods of 8 hours, 18 hours, and natural daylength in the greenhouse. The 8-hour photoperiod comprised only natural daylight; the 18-hour one consisted of all the natural daylight plus Mazda light of 150 foot-candles for the remaining hours of the photoperiod. The fourth lot, of 56 plants, was planted in the field and subjected to natural daylength. The 18-hour and natural-daylength lots in the greenhouse were grown in soil beds, while the 8-hour lot was grown in 12-inch pots. By June 21, 1945, 34 of the 110 plants had produced macroscopic flower buds. These 34 plants were distributed in approximately equal proportions among the groups on the different photoperiods. The position of the first flower buds for these 34 plants averaged only two to three nodes higher than in the last lot of F_2 plants dissected on May 23. Since the terminal bud of castor bean includes an average of five to six nodes, the flower buds observed on June 21 must have been present as flower primordia on May 23 or earlier and thus were evidently initiated before the differential photoperiod treatments were begun.

A total of seventy-six F_2 plants remained in the four different lots after June 21. None was observed to develop macroscopic flowers prior to September 17, when they were harvested and dissected. On dissection twenty-one plants were found to have microscopic flowers. Fifteen were from the natural-daylength lot in the field and the other six from the 8-hour lot in the greenhouse. These data suggest that the differential photoperiod treatments had no outstanding effects on flowering of F_2 individuals.

Six of the plants that were flowerless at the September 17 dissection were transplanted on October 9 to a greenhouse. They were maintained on a 17-

hour photoperiod until March 20, 1946, without forming flower buds or primordia, although they produced an average of fifty-four nodes each during that period. Apparently such F_2 plants when grown in the field locally are vegetative at time of frost for reasons other than need of a longer growing season.

The flowering data for the parent, F_1, and F_2 populations planted on February 1, 1945, are summarized in table 3. All parent and F_1 plants flowered but only

TABLE 3

FREQUENCY DISTRIBUTION WITH RESPECT TO NODE NUMBER AT WHICH FIRST FLOWERING OCCURRED FOR TWO VARIETIES OF CASTOR BEAN AND THEIR F_1 AND F_2 PROGENIES. EXPERIMENT IV*A*. PLANTED IN GREENHOUSE FEBRUARY 1, 1945. FINAL DATA COLLECTED SEPTEMBER 17, 1945

Location of first flowers (node no.)	Character of population									
	Parent		F_1	F_2						
	Ky. 38	D.P. 227	X_3	$X_{3\text{-}5}$	$X_{3\text{-}6}$	$X_{3\text{-}7}$	$X_{4\text{-}3}$	$X_{4\text{-}4}$	$X_{4\text{-}5}$	Total
6	.....	6	.....	1	.....	.....	.....	.....	.....	1
7	.....	40	1	27	6	7	4	2	8	54
8	.....	14	4	42	10	15	16	4	17	104
9	.....	.....	6	17	1	7	9	5	5	44
10	.....	.....	1	21	2	5	10	13	4	55
11	7	.....	1	24	5	6	23	2	11	71
12	6	.....	4	9	3	3	8	1	7	31
13	12	.....	2	6	3	2	6	1	3	21
14	6	.....	.....	4	.....	2	3	2	1	12
15	2	.....	.....	5	1	4	3	2	2	17
16	1	.....	.....	4	.....	1	3	4	1	13
17	.....	.....	.....	2	1	1	2	2	3	11
18	.....	.....	.....	5	1	1	1	.....	.....	8
19	.....	.....	.....	2	.....	1	3	1	.....	7
20	.....	.....	.....	4	.....	2	2	1	1	10
21	.....	.....	.....	3	.....	1	3	1	1	9
22	.....	.....	.....	1	.....	.....	2	.....	.....	3
23	.....	.....	.....	.....	.....	.....	1	.....	.....	1
24	.....	.....	.....	1	.....	.....	.....	.....	1	2
25	.....	.....	.....	2	1	.....	.....	.....	.....	3
26	.....	.....	.....	3	.....	.....	.....	.....	.....	3
27	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	.....	.....	.....	1	.....	.....	.....	.....	.....	1
29	.....	.....	.....	1	.....	.....	1	.....	.....	2
30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
31	.....	.....	.....	1	.....	.....	.....	.....	1	2
32	.....	.....	.....	.....	.....	.....	.....	.....	1	1
33	.....	.....	.....	1	.....	.....	.....	.....	.....	1
34	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
35	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
36	.....	.....	.....	.....	.....	1	.....	1	.....	2
37	.....	.....	.....	.....	.....	1	.....	.....	.....	1
38	.....	.....	.....	1	.....	.....	.....	.....	.....	1
Flowerless	0	0	0	21	0	12	14	3	5	55
Total	34	60	19	209	34	72	114	45	72	546
% flowerless	.....	.....	.....	10.0	0.0	16.7	12.3	6.7	6.9	10.1
Av. fl. node	12.8± 0.2*	7.1± 0.1*	9.9± 0.4	11.6	10.5	11.7	12.1	12.4	11.3	11.7

* For parent populations, significantly different from each other and each from F_1 at the 1% level.

89.9% of the F_2 plants. The percentage of flowerless F_2 plants in this study is similar to the 8% obtained in the field (5) on F_2 material from the same parents.

The average node positions of first flowers for the parents, Kentucky 38 and D.P. 227, were significantly different from each other and from the average position in the F_1 population, which was intermediate between the two parents.

In only one of the six F_2 populations, namely, X3-6, which was the smallest one, did all plants flower or form primordia. Among the remaining five lines, the percentages of flowerless plants ranged from 6.7 to 16.7. Disregarding the flowerless plants, none of the F_2 populations differed from one another with respect to the average node position of the first flower primordia. The greatest number of nodes per plant on the flowerless individuals dissected in September was 45; the least, 33.

On similar material planted in the field during 1944, DOMINGO (5) observed an average of 8, 10, 10, and 10 nodes for the position of first flower primordia on plants of Kentucky 38, D.P. 227, F_1, and F_2, respectively. The present data for F_1 and F_2 approximate his results, but the results for the parents were outstandingly different. In the earlier data (5) the parental means differed by two nodes, D.P. 227 having the greater mean. In the present study the mean for Kentucky 38 was five nodes greater than that for DP. 227. A possible explanation for these differences in results is considered in reporting subsequent experiments.

EXPERIMENT IV*B*.—At approximately monthly intervals following the planting of February 1, 1945, four additional plantings of both Kentucky 38 and D.P. 227 parent seed were made in 8-inch pots in the greenhouse. These plants were likewise grown on $14\frac{1}{2}$-hour photoperiods until May 6, when the natural daylength exceeded $14\frac{1}{2}$ hours, and all plants were put on natural photoperiod alone. The last two monthly plantings were grown only with natural photoperiod, since the natural daylength was already in excess of $14\frac{1}{2}$ hours.

The average node position of first flowers on Kentucky 38 in the February 1 planting was 12.8 (table 4), which was four nodes higher than in experiment I. With each successive planting of this variety, the first flowers were formed at either the same or at a lower mean position. The mean positions of first flowers for each of the first two plantings were significantly higher than those of each of the last two plantings. These differences may have been correlated with the fact that the last two plantings had received solely natural daylight which exceeded $14\frac{1}{2}$ hours after May 6, while the earlier lots received a fixed $14\frac{1}{2}$-hour photoperiod, a portion of which was Mazda light.

The results for D.P. 227 for the five planting dates were in contrast to those obtained with Kentucky 38 in both the degree and the direction of change of the node position of first flowers. With D.P. 227 there was a substantial increase in this mean node value with the successive planting dates. These increases were all statistically significant at the 1% level of probability. In the case of the parents planted on February 1, 1945, the average node position of first flowers for Kentucky 38 plants was higher than for D.P. 227 plants (table 4). This relationship was the reverse of what DOMINGO (5) reported for these varieties grown in the field during the summer. However, in the case of the fifth planting made on May 26, 1945, in the greenhouse, the relative location of the first flower node in the two varieties was the reverse of

that obtained with the February lots and was then like that reported for the field-grown material (5), with first flowers of D.P. 227 plants at a higher node position than were those of Kentucky 38. This differential trend in node values for these varieties took place simultaneously with changes in duration of natural daylight occurring through the growing period.

whether flowerless F_2 plants could be made to flower if grafted to those of other types which flower readily. It is known in the case of soybeans that plants of the Biloxi variety, which are vegetative with long photoperiods, flower readily on such photoperiods if grafted to those of the Agate variety which normally flower on long daylengths (7).

TABLE 4

FREQUENCY DISTRIBUTION WITH RESPECT TO NODE OF FIRST FLOWERING FOR TWO VARIETIES OF CASTOR BEAN PLANTED ON FIVE DIFFERENT DATES. PLANTS GROWN IN GREENHOUSE ON 14½-HOUR PHOTOPERIOD UNTIL MAY 1, 1945, AND SUBSEQUENTLY ON NATURAL PHOTOPERIOD. EXPERIMENT IV*B*

Planting date	Node at which first flower primordia developed											Total plants	Average
	6	7	8	9	10	11	12	13	14	15	16		
	Kentucky 38												
2– 1–45	....	....	....	....	....	7	6	12	6	2	1	34	12.8±0.2*
3– 3–45	....	....	....	5	5	2	6	8	21	7	1	55	12.9±0.2*
4– 2–45	....	....	....	2	5	7	10	12	10	3	....	49	12.4±0.2
5– 1–45	....	....	....	....	6	10	14	9	3	....	....	42	11.8±0.2
5–26–45	....	....	....	2	9	7	8	7	2	....	4	39	11.9±0.3
	D.P. 227												
2– 1–45	6	40	14	....	....	....	....	....	....	....	....	60	7.1±0.1†
3– 3–45	....	7	25	10	2	....	....	....	....	....	....	44	8.2±0.1†
4– 2–45	....	1	14	28	11	....	2	....	....	....	....	56	9.0±0.1†
5– 1–45	....	....	2	16	21	8	1	....	....	....	....	48	9.8±0.1†
5–26–45	....	....	....	....	....	....	11	12	9	3	....	35	13.1±0.2†

* Significantly different at the 1% level from 5–1–45 and 5–26–45 plantings of Kentucky 38.
† Significantly different at the 1% level from all other plantings of D.P. 227.

EXPERIMENT IV*C*.—A number of reciprocal grafts were made involving apparently flowerless F_2 individuals planted on February 1, 1945, and plants of the Kentucky 38 parent. The F_2 plants were grown in a soil bed in the greenhouse on 18-hour photoperiod. The Kentucky 38 plants were planted in pots on May 1, 1945, and had been growing vegetatively with an 8-hour photoperiod. The purpose of the grafts was to determine

The first bud grafts were made on June 30, 1945. In all such grafts lateral buds were employed. With both stock-scion combinations the terminal bud and all but one lateral bud above the location of the graft were removed from the stock. Most of these grafts were successful, and satisfactory growth of the scion occurred in all such cases (fig. 2*A*). In one instance an approach graft was made, a flowerless F_2 plant serving final-

ly as the stock and a Kentucky 38 plant as the scion (fig. 2*B*).

In all cases the Kentucky 38 plant flowered, and the flowerless F_2 plant remained vegetative, regardless of the variety used as stock or scion. In all cases the first flower primordia to appear on Kentucky 38, whether serving as stock or scion, were developed at a substantially higher node number than could be accounted for by the number of nodes present in the initial bud.



FIG. 2.—Grafts between Kentucky 38 variety parent and a flowerless F_2 plant, the former serving as scion and the latter as stock. *A*, bud graft with F_2 stock vegetative and the scion showing young inflorescence. *B*, approach graft with F_2 stock vegetative and the scion showing elongated inflorescence. (Photographed September 14, 1945.)

EXPERIMENT V

Plants of Kentucky 38 did not flower when grown in the greenhouse during winter periods in which the natural daylength was shortest and frequently of lowest average light intensity, nor did they flower during these periods when incandescent light of 25 foot-candles intensity supplemented the natural daylength to provide 24-hour photoperiods. However, during the months of March through August all the Kentucky 38 plants flowered, and the first flowers were formed at a lower average node position on those that were grown during the middle of this period when the longest natural daylengths occurred. Such data suggest that flowering of this species may be influenced both by length of photoperiod and by total radiation received during the photoperiod. The influence of these two factors was therefore investigated further.

EXPERIMENT V*A*.—In this experiment the factors of duration and intensity of light were investigated for Kentucky 38 and D.P. 227 at a time of year in which both varieties had previously flowered. On July 21, 1945, seed was planted outdoors in four soil beds with twenty plants of each variety in each bed. Two beds were subjected to full natural daylength, the other two beds to 9 hours of natural daylight. Differential photoperiods were begun immediately with germination of seed and were

maintained until September 5, 1945, at which time node counts were made (table 5). All plants flowered when subjected to 9-hour and to natural photoperiods; but the Kentucky 38 plants flowered at a significantly higher node position with the 9-hour photoperiod than with natural photoperiod, whereas the reverse relationship was found among the D.P. 227 plants.

EXPERIMENT *VB*.—The effect of several different light intensities upon the earliness of flowering of Kentucky 38 variety was measured by the node number at which first flowers developed. On August 18, 1945, seed of Kentucky 38 was planted outdoors in six soil beds following the procedure used in experiment *VA*. Light intensities of 100, 70, and 30% of that available naturally were applied in duplicate, the lower intensities being obtained by shading with different numbers of layers of tobacco cloth.

The plants were grown until October 3, 1945, at which time either the node position of first flowers or the total number of nodes on nonflowering plants was recorded for each plant. Duplicate fresh-weight and quadruplicate dry-weight data were obtained on tops cut at the cotyledonary node (table 6).

Obviously significant differences in the flowering responses resulted with the different treatments. No plants flowered when grown with 30% of full light intensity, although they had produced an average of 14.2 nodes per plant. With 70% intensity, fifty-two of the sixty-two plants flowered with first flowers at the average node position of 10.4. The nonflowering plants developed an average of 15.5 nodes in their main stems. All plants flowered when grown with 100% of the natural light available, and the average node at which first flowers occurred was 9.0. The difference in the average position of first flowers was statistically significant. The same was true for total nodes on the nonflowering plants receiving the two lowest intensity treatments.

Statistically significant differences in average fresh-weight and in dry-weight percentage were found among plants that received different intensities. The highest average fresh weight occurred with the 70% intensity, the lowest with the 30% intensity. The presence of the highest average fresh weight in plants of the 70% lot may possibly be accounted for by the differences in flowering response between this lot and the 100% one. The delayed flowering response obtained with

TABLE 5

EFFECT OF PHOTOPERIOD UPON INITIATION OF FLOWER PRIMORDIA IN KENTUCKY 38 AND D.P. 227 VARIETIES OF CASTOR BEAN GROWN IN OUTDOOR SOIL BEDS. EXPERIMENT *VA*. PLANTED JULY 21, 1945. HARVESTED SEPTEMBER 5, 1945. FORTY PLANTS PER LOT

VARIETY	AVERAGE NODE AT WHICH FIRST FLOWER PRIMORDIA OCCURRED	
	Natural daylength	9-hour photoperiod
Kentucky 38	8.7±0.1*	12.0±0.1
D.P. 227	9.7±0.1	8.9±0.1*

* These two lots are not significantly different from each other. All other differences significant at the 1% level of probability.

plants of the 70% lot in contrast to the 100% lot, plus the fact that a number of the former were still vegetative at harvest, could account for its greater fresh weight.

Discussion

Relatively little has been reported concerning the effects of either duration of photoperiod or intensity of light upon the flowering of castor bean. ADAMS (1, 2) observed the effect of these two factors upon the flowering of several species of plants including the castor bean but did not name the variety of castor bean employed. In one of his experiments with light intensity all plants flowered when grown solely with continuous light from 300-watt or 500-watt nitrogen-filled lamps. Investigation of the effects of duration of light (2) was confined to extremely short photoperiods, not exceeding 3 hours of natural light daily. The results of his study were not conclusive, since approximately half of the plants of each photoperiod lot failed to flower at the end of a 3-month growing period.

The present studies indicate that both duration of photoperiod and light intensity are factors which may influence formation of flower primordia in certain varieties of castor bean. This species thus seems to be different from Biloxi soybean (4) and *Xanthium pensylvanicum* (6), which are little influenced in flowering by rather wide differences in total radiant energy but are very sharply influenced by small differences in daylength. These plants remain vegetative if grown on photoperiods that exceed a certain critical length and flower promptly if grown on shorter ones. The intensity of supplemental light used to extend the natural photoperiod, and so prevent flowering of such plants, need not be more than 25 foot-candles and can be as low as a fraction of 1 foot-candle.

With plants of Kentucky 38 variety, formation of flower primordia is apparently dependent upon a total light-energy factor, and thereby both duration and intensity of light are involved. Measured in terms of node number at which first flowers were formed, the earliest flowering occurred when plants were grown on photoperiods of natural length or longer, during the spring, summer, and fall months, a period of the year having longest natural daylengths and relatively high average daily light intensity. Plants grown during these same periods on 8-

TABLE 6

EFFECT OF LIGHT INTENSITY UPON PLANTS OF KENTUCKY 38 VARIETY CASTOR BEAN GROWN IN OUTDOOR SOIL BEDS ON NATURAL PHOTOPERIOD. EXPERIMENT *VB*
PLANTED AUGUST 18, 1945. HARVESTED OCTOBER 3, 1945

LIGHT INTENSITY (%)	NO. OF PLANTS	AV. FRESH WEIGHT (GM.)	DRY WEIGHT* (%)	FLOWERING PLANTS		VEGETATIVE PLANTS	
				No.	Av. first flower node	No.	Av. total no. of nodes
100.....	57	76.8±2.3†	10.57±0.5	57	9.0±0.02‡	0	.....
70.....	62	97.8±0.8	10.03±0.4	52	10.4±0.02	10	15.5±0.2‡
30.....	52	34.1±0.9§	9.48±0.5	0	.....	52	14.2±0.1

* Figures are averages, each derived from four 200-gm. fresh-weight samples.
† Significantly different from 70% intensity lot at the 5% level.
‡ For average first flower node and average number of nodes, significantly different from other treatment at the 1% level.
§ Significantly different from other two lots at the 1% level.

hour natural photoperiod remained vegetative. Likewise plants grown during the winter months of shortest natural daylengths and low average daily light intensity failed to form flower primordia with any duration of daily photoperiod or even with continuous light. During this period of the year extension of the natural photoperiod with incandescent-filament light of 150 foot-candles was not effective in promoting floral initiation. However, when plants were grown for 7 weeks during winter months with 15-hour or longer photoperiods in a controlled-environment room in which the light intensity was about 2000 foot-candles, flower primordia were formed on ten of a total of twenty-eight individuals.

These data indicate that a relatively high level of light energy is a major requirement for floral initiation in plants of Kentucky 38 variety. It was not possible to determine the quantity of light energy required, but, in terms of duration of photoperiod and intensity of light, several of the combination requirements have been established for definite seasons of the year. D.P. 227 differed from Kentucky 38 variety in that it flowered regardless of duration of photoperiod on which it was grown. With photoperiods conducive to flowering, first flowers in Kentucky 38 occurred at successively lower node positions as the daylength increased, while the reverse trend was true for first flowers of D.P. 227 plants grown with these daylengths.

All attempts to induce formation of flower primordia on flowerless F_2 plants resulting from crosses of Kentucky 38 and D.P. 227 parents were unsuccessful. On the basis of the flowering responses of the parents to different photoperiods and light intensities, as well as the prompt flowering of F_1 individuals, it does not appear probable that the flowerless F_2 plants lack the capacity to form flowers. More likely other factors of the environment, such as temperature and nutrition, may be involved in controlling formation of flower primordia, along with the light-energy factor effective in the case of the Kentucky 38 and D.P. 227 parents. Significantly higher intensities of light occur in tropical regions, to which castor bean is indigenous, than in temperate regions. ALLEE (3) reported maximum light intensities of the order of 20,000 foot-candles in a series of observations over several weeks at Barro Colorado Island in Panama as compared with 14,000 at Chicago, Illinois; but a great many variables are involved, and these figures admittedly are not a basis for exact comparison. It may be possible that the flowerless F_2 plants require even higher light intensities than do Kentucky 38 plants—intensities such as prevail in tropical regions.

Summary

1. Studies were made of the effects of photoperiod and light intensity upon flower formation in plants of several genetically different populations of castor bean. Plants were grown in soil in the greenhouse, field, or controlled-environment rooms. The node position of the first flowers and the total number of nodes on nonflowering plants were the criteria used for measuring the responses of plants to duration and intensity of light.

2. In experiments concerned with photoperiod alone, marked variation in degree of flowering response was observed for a number of varieties. Some failed to flower on either long or short photoperiods, while those of other varieties flowered on both photoperiods. In

the case of one variety, flowers developed only on plants grown with long photoperiod.

3. Plants of two parents and their F_1 and F_2 progenies, the last of which contained plants that remain flowerless when grown under normal field conditions at Beltsville, Maryland, were grown with controlled photoperiod. Plants of both parents and the F_1 progeny flowered at relatively low node positions, the F_1 plants being intermediate between the parents in this character. The node position of first flowers on most of the F_2 plants was within the range of that of the parents and the F_1 progeny, but 10% of the F_2 plants remained vegetative under the several treatments given.

4. Flowerless F_2 plants remained vegetative when grown with several different photoperiods and likewise when grafted either as stock or as scion with vegetative parent plants which subsequently were induced to flower.

5. Earliness of flowering of parent plants whose F_2 populations contain the flowerless character was significantly affected by variation in photoperiod or in light intensity. In the case of one parent, flowering was markedly controlled by a light-energy factor. In this case floral initiation occurred only on long photoperiods applied during spring, summer, and fall. During the short days of winter, flowers failed to form even under a continuous 24-hour photoperiod in which supplementary artificial illumination was supplied.

LITERATURE CITED

1. ADAMS, J. Duration of light and growth. Ann. Bot. 38:509–523. 1924.
2. ———. Some further experiments on the relation of light to growth. Amer. Jour. Bot. 12:398–412. 1925.
3. ALLEE, W. C. Measurement of environmental factors in the tropical rain forest of Panama. Ecol. 7:273–302. 1926.
4. BORTHWICK, H. A., and PARKER, M. W. Photoperiodic responses of several varieties of soybeans. BOT. GAZ. 101:341–365. 1939.
5. DOMINGO, W. E. Flowerless castor bean plants. Jour. Hered. 36:116–120. 1945.
6. HAMNER, K. C., and BONNER, J. Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1938.
7. HEINZE, P. H.; PARKER, M. W.; and BORTHWICK, H. A. Floral initiation in Biloxi soybean as influenced by grafting. BOT. GAZ. 103:518–530. 1942.
8. PARKER, M. W. Environmental factors and their control in plant experiments. Soil Sci. 62:109–119. 1946.

INFLUENCE OF POTASSIUM AND SODIUM ON METABOLISM OF PEANUT COTYLEDONS DURING GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 585

HUBERT J. DYER

Introduction

It had been noticed that, when cocklebur was grown under potassium-deficient conditions, the cotyledons persisted in a fleshy and turgid condition much longer than did those of control plants. It was conjectured that this persistence might be a result of impedance of the vascular transport of food reserves from the cotyledons, since both breakdown (15) and interference with formation of phloem tissue (26) have been described as potassium-deficiency symptoms.

An experiment was designed to investigate the relative rates of removal of the important cotyledonary food reserves

under conditions of adequate (+K) versus no (−K) potassium supply, as well as to study other symptoms associated in the −K syndrome. Since seeds of cocklebur were unavailable in large quantities, Virginia peanut seed was chosen as the test object. It has the advantages of being large, fatty, and epigeal in germinating habit. The experiments were carried out in the dark to avoid confusion of the interpretation by the addition of new products of photosynthesis.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS USED

Salts (Stock solutions)	Solution A (+K−Na)	Solution B (−K+Na)	Solution C (−K−Na)
$Ca(NO_3)_2$ 0.05M	20 ml./l.	20 ml./l.	20 ml./l.
$MgSO_4$ 0.05M	10 ml./l.	10 ml./l.	10 ml./l.
KH_2PO_4 0.05M	10 ml./l.	.....	.....
NaH_2PO_4 0.05M	.....	10 ml./l.	.....
$Ca(H_2PO_4)_2$	.....	.....	(0.0025M)
H_3BO_3 0.0463M, $MnCl_4$ 0.0064M, $ZnSO_4$ 0.00076M, $CuSO_4$ 0.0003M, H_2MoO_4 0.0004M	1 ml./l.	1 ml./l.	1 ml./l.
$Fe_2(C_4H_4O_6)_3$ 0.0089M	1 ml./l.	1 ml./l.	1 ml./l.
Atmospheres	1.12	1.12	1.05

Material and methods

In each experiment Virginia peanuts obtained from a commercial seedsman were shelled, examined, and selected for undamaged seeds. They were planted in washed quartz sand in glazed pots, the drains of which were covered with glass wool. Each pot was watered before and after planting and thereafter daily or every second day, depending on the experiment, with its appropriate nutrient solution. Nutrient solutions (table 1) were based on those suggested by HOAGLAND and ARNON (9). The pots were kept in a basement room of relatively even temperature, and the room was maintained in complete darkness except at times of watering and harvest. These operations were carried out by the light of a 15-watt blue bulb shielded except at the tip and carried in the hand. The plants were grown for 6 (experiment I) or 7 (experiments II and III) and for 15 days (all experiments).

At harvest the sand was washed from the root systems. It was found impossible, however, either to retain all the brittle lateral roots of the 15-day plants or to remove the sand from the roots completely by washing and rinsing with concentrated sodium chloride solution. Therefore, the root systems of the 15-day plants were severed from the hypocotyls at the point of divergence of the first lateral root and were discarded.

Immediately at harvest the wet weights of the 6- or 7-day cotyledons and axes (hypocotyl plus epicotyl) and of the 15-day cotyledons, hypocotyls, and epicotyls were taken separately. The axial tissues were dried in an air-blast oven at 150° C. for 5 minutes; then the temperature was reduced to 70° C. to complete the drying. After drying for 4 hours, they were placed in desiccators and held until weighed. The fresh cotyledons from each treatment were harvested in triplicate lots, weighed in pairs, and the lot members mixed together. Each lot was ground

in a meat-chopper with 2 gm. of calcium carbonate for each 100 gm. of tissue in such a manner that the discharge from the mill dropped into boiling redistilled ethanol in quart mason jars. The jars were sealed and held in a dark cabinet until the contents could be analyzed.

Methods for chemical analyses were those described by LOOMIS and SHULL (13), except that lipids were determined by the method of SANDO (20) and lipolytic activity was estimated by the method of LONGENECKER and HALEY (12) on fresh cotyledons. Invert sugars and crude fiber were both determined by difference (total sugar − reducing sugars = invert sugars; total dry matter − dry weight of determined substances = crude fiber). Comparisons of wet-weight and dry-weight data were made by the analysis of variance technique described by SNEDECOR (23).

Material for histological study was killed in CROOKS's modification of Navashin's solution (3), washed in tap water for 48 hours, dehydrated in a tertiary butanol–ethanol series, imbedded in paraffin, sectioned at 15 μ, and stained with Flemming's triple stain.

DESCRIPTION OF EXPERIMENTS

In experiment I, selected peanut seeds were further selected by rejecting all seeds falling outside the limits of ± 0.05 gm. of the mean seed weight. The seeds were planted as described above, and either solution A or solution B (table 1) was applied daily. Three lots of each treatment were harvested at 6 days after planting and three more lots at 15 days. Analyses were made for lipids on the absolute basis only.

Experiment II was performed similarly to experiment I, except that the first harvest was taken on the 7th rather than on the 6th day after planting. Analyses were made of these samples of cotyledons for moisture, total sugars, reducing sugars, starch and dextrins, acid-hydrolyzable substances, soluble nitrogen, insoluble nitrogen, and total lipids.

Experiment III was designed similarly to experiments I and II, with the following exceptions: three instead of two nutrient solutions were used: A, B, and C (table 1); pots were treated with nutrient solution every second day; and the seeds, instead of being merely selected for size, as in experiments I and II, were, in addition, individually weighed and their weights recorded. Solution C was designed to furnish the same amount of $H_2PO_4^-$ ions as solutions A and B, to supply neither potassium nor sodium, to cause as small a difference as possible from the other two solutions in the concentration of calcium, and to have approximately the same ionic concentration as the other solutions. Plants were again harvested at 7 and at 15 days after planting. In this experiment wet weights of the 7-day cotyledons and axes and of the 15-day cotyledons, hypocotyls, and epicotyls were taken; dry weights of 7-day axes and of 15-day hypocotyls and epicotyls were also recorded. Dry weights of cotyledons could not be obtained because of the analytical procedure used.

Results

Removal of the two predominant complex reserve materials, fats and proteins, from the cotyledons proceeded somewhat slowly for the first 6 or 7 days after planting but went on much more rapidly during the next 8 days (fig. 1). It is assumed from the data (tables 2–4) and from the fact that cotyledons of etiolated seedlings were not visibly necrotic until 4 weeks after planting that the quantity of lipid and protein reserves remaining in peanut cotyledons during germination

and early growth follows an inverse sigmoid curve with time the abscissa. The effect of substitution of sodium for potassium in the nutrient substrate appears to be to flatten this curve; this is most marked at the 7-day point. Wider differences are evident between treatments in dry weight of cotyledons, dry weight of entire plants, and grams of lipid and protein per 100 plants at the mid-point of the experimental period than at the end. The 7-day plants had less dry matter both in axes and in cotyledons, and less lipid and protein in the cotyledons under −K +Na conditions (solution B) than under +K −Na (solution A). Although carbohydrates in the cotyledons, especially starch, increased somewhat during germination, the increase was not in the same ratio in experiments II and III, nor was it consistent with treatment. There was a reversal between the 7th and 15th day in the distribution of lipids between treatments in all three experiments: whereas more lipid remained in the +K −Na cotyledons at 7 days, by the

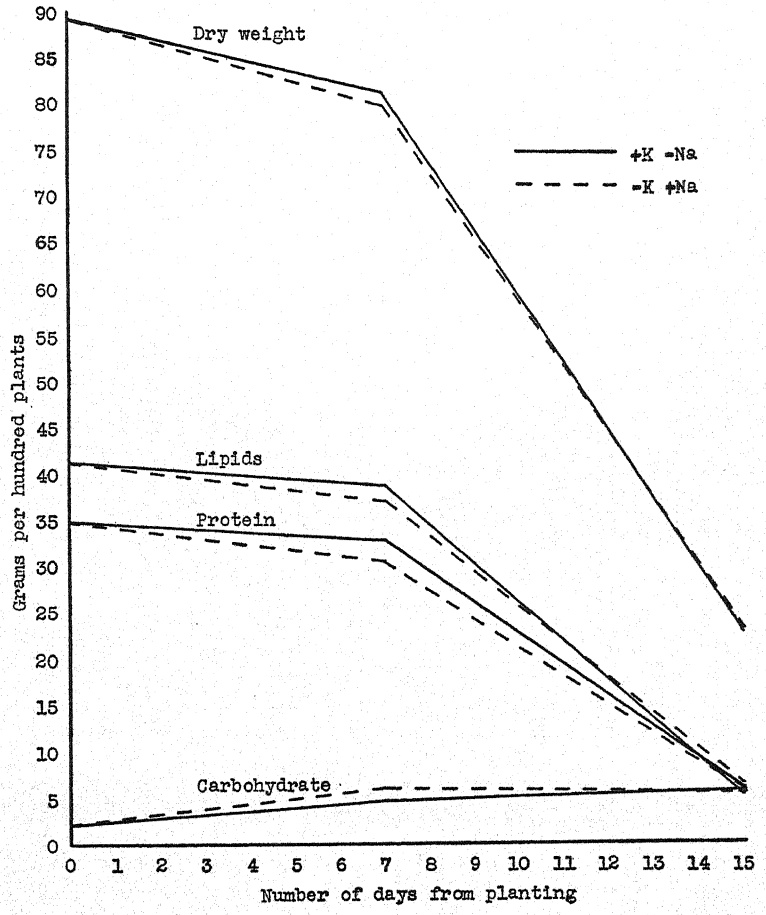


FIG. 1.—Changes in amount of dry weight, carbohydrates, lipids, and proteins in cotyledons of Virginia peanuts during first 15 days after planting in dark as affected by substitution of sodium for potassium (data of experiment II).

15th day they had lost more lipid than the −K +Na cotyledons. When both potassium and sodium were eliminated from the nutrient solution (solution C, experiment III), lipids were not so rapidly lost as when either was present, and the amounts of starch found in the cotyledons more nearly approximated those for the +K −Na treatment.

Statistical analysis of the wet- and dry-weight data for 15-day plants from experiment III (table 4) revealed highly significant differences owing to treatment between wet epicotyl weights, between wet weights of entire plants (without roots), and between dry epicotyl weights. Differences significant at the 5% point were found between wet weights of axes (without cotyledons and roots), between dry weights of axes, and between wet weights of cotyledons. Differences between treatments of original seed weights were not significant, nor were differences between dry weights of 15-day hypocotyls, nor between any wet or dry weights of parts or complete plants at 7 days.

There was no visible difference among the roots of plants from the various treatments. Histological study of the vascular tissue of the cotyledonary petiole revealed no evidence of breakdown and no differences between treatments in amount of phloem present. Tests of lipolytic activity performed *in vitro* on fresh cotyledons using peanut oil as a substrate showed no differences in fat-hydrolyzing rate between treatments.

TABLE 2

ANALYTICAL DATA, EXPERIMENTS I AND III, GRAMS PER 100 PLANTS

	Experiment I	Experiment III			
	Total lipids	Dry weight	Moisture	Starch and dextrins	Total lipids
Seeds	36.31	89.01	18.52	0.92	41.26
6- or 7-day cotyledons:					
+K−Na	18.87	76.37	68.27	4.72	26.96
−K+Na	17.78	70.72	77.06	3.59	22.26
−K−Na	.....	74.77	73.83	4.63	27.58
15-day cotyledons:					
+K−Na	3.83	35.90	55.37	2.70	6.18
−K+Na	4.62	37.05	58.63	2.42	6.33
−K−Na	.....	36.79	60.34	2.93	7.33

Discussion

When potassium was lacking from the nutrient medium during germination of Virginia peanuts, certain well-known potassium-deficiency symptoms became recognizable as early as the 7th day after planting. It is especially interesting that these symptoms were induced in such a short time in a seedling which, according to analyses cited by WINTON and WINTON (27), contains 35–40% K_2O in the ash of the seed.

In the following discussion of the condition and metabolism of the cotyledons the writer has placed more emphasis on the middle part of the period covered by the experiments (although not to the exclusion of the later conditions); it is thought that their metabolism during

TABLE 3

ANALYTICAL DATA, EXPERIMENT II

	Dry weight	Moisture	Reducing substances	Invert sugars*	Starch and dextrins	Acid-hydrolyzables	Soluble nitrogen	Insoluble nitrogen	Total protein	Total lipid	Total carbohydrate	Crude fiber†
Seed:												
% of dry weight		17.22‡	0.02	1.15	1.10	None	0.06	6.28	39.26	48.95	2.27	9.46
Gr./100 plants	89.01	18.52	.02	0.97	0.92	None	0.05	5.59	34.94	41.26	1.91	8.42
7-day cotyledons (+K−Na):												
% of dry weight		50.16‡	.08	1.70	4.01	None	0.05	6.44	40.23	47.19	5.79	6.74
Gr./100 plants	81.79	82.33	.06	1.40	3.28	None	0.04	5.26	32.90	38.60	4.74	5.51
7-day cotyledons (−K+Na):												
% of dry weight		51.23‡	.10	1.82	5.38	None	0.05	6.17	38.55	46.41	7.30	7.69
Gr./100 plants	79.98	83.99	.09	1.53	4.30	None	0.04	4.93	30.83	37.12	5.92	6.15
15-day cotyledons (+K−Na):												
% of dry weight		78.33‡	.40	4.29	19.65	0.39	1.12	4.02	25.10	22.06	24.73	26.99
Gr./100 plants	22.82	82.50	.09	0.98	4.48	0.09	0.26	0.92	5.73	5.03	5.64	6.16
15-day cotyledons (−K+Na):												
% of dry weight		76.50‡	.23	3.67	18.02	0.33	0.96	4.02	24.79	27.16	22.25	24.84
Gr./100 plants	23.10	75.20	0.05	0.85	4.16	0.07	0.22	0.93	5.81	6.27	5.13	5.74

* Determined by difference: total sugars − reducing sugars = invert sugars.

† Determined by difference: total dry matter − total dry weight of determined substances = crude fiber.

‡ Per cent of total weight.

TABLE 4

WET AND DRY WEIGHT DATA, EXPERIMENT III

Material	Treatment	Mean weight (gm.)	Standard deviation	F	Degrees of freedom
*7-day plants:**					
Original seeds, air-dry	+K−Na	0.869	0.072	1.315	2, 105
	−K+Na	0.860	.067		
	−K−Na	0.889	.086		
Cotyledon pairs, wet	+K−Na	1.431	.133	1.376	2, 105
	−K+Na	1.444	.128		
	−K−Na	1.469	.137		
Axes plus cotyledons, wet	+K−Na	3.006	.279	1.151	2, 105
	−K+Na	2.928	.260		
	−K−Na	2.986	.230		
Axes alone, wet	+K−Na	1.551	.251	1.076	2, 105
	−K+Na	1.472	.220		
	−K−Na	1.509	.211		
Axes alone, air-dry	+K−Na	0.160	.098	2.329	2, 105
	−K+Na	0.146	.024		
	−K−Na	0.152	.026		
15-day plants:†					
Original seeds, air-dry	+K−Na	0.858	.092	2.105	2, 97
	−K+Na	0.898	.104		
	−K−Na	0.858	.075		
Cotyledon pairs, wet	+K−Na	0.849	.183	4.509‡	2, 97
	−K+Na	0.941	.216		
	−K−Na	0.991	.164		
Axes (without roots) plus cotyledons, wet	+K−Na	5.615	.796	7.066§	2, 97
	−K+Na	5.292	.772		
	−K−Na	4.906	.578		
Axes without roots or cotyledons, wet	+K−Na	4.721	.671	4.760‡	2, 97
	−K+Na	4.374	.853		
	−K−Na	4.067	.929		
Axes without roots or cotyledons, air-dry	+K−Na	0.422	.066	4.692‡	2, 73
	−K+Na	0.401	.052		
	−K−Na	0.369	.067		
Epicotyls, wet	+K−Na	2.402	.428	10.899§	2, 97
	−K+Na	2.139	.388		
	−K−Na	1.872	.370		
Epicotyls, air-dry	+K−Na	0.229	.046	6.325§	2, 93
	−K+Na	0.215	.034		
	−K−Na	0.195	.032		
Hypocotyls, without roots, air-dry	+K−Na	0.186	.039	1.933	2, 77
	−K+Na	0.187	.034		
	−K−Na	0.176	0.044		

* Based on thirty-six plants in each treatment.

† Based on twenty-three to thirty-five plants in each treatment.

‡ Significant at odds of 19:1. § Significant at odds of 99:1.

this earlier period was more free than during the later period from the effects of the hunger metabolism which supervenes as the cotyledonary reserves become exhausted and which might be expected to mask the effects of the treatments (22).

DRY MATTER.—Dry weights of cotyledons and plant axes at 7 days were less under −K −Na and much less under −K +Na conditions than in the controls (tables 2–4). This is interpreted as confirming the frequently reported symptom of potassium deficiency—exalted respiratory rate (7, 19, 25). In addition, it indicates that the presence of large amounts of available sodium in the absence of much potassium induces a higher respiratory rate than is shown by plants deprived of both sodium and potassium. Such a situation has not come to the writer's attention through the literature. This differential in loss of dry weight between treatments is paralleled by a differential in rates of utilization of lipid and protein reserves.

Dry weights of cotyledons at 15 days were least under +K −Na and greatest under −K +Na conditions; this apparently is analogous to the situation earlier observed in cocklebur. At the same time, weights of plant axes and entire plants were greatest under +K −Na and least under −K −Na conditions. It is thought that this change in relationships compared with the status at 7 days was due to the change in character of growth between the 7th and 15th days; during this period the entire lateral root system of the seedling was developed. At the 7th day the primary root was short, smooth, and branchless, while at the 15th day its upper half was covered with laterals varying in length from 3 to 4 cm. at the base of the hypocotyl to mere swellings at the mid-length of the primary root. The development of so many additional terminal meristems would impose a seriously accelerated demand on the potassium reserves of the seed (5, 15, 21); it could thus bring on not only the reversal of relative respiratory rates between the two treatments but the stunting of the epicotyls of the −K −Na plants in comparison with the other two (+K −Na and −K +Na) treatments. In the −K +Na plants the situation is not so severe, probably because of the effectiveness of sodium in partially relieving the potassium deficiency (1, 8, 11).

Moisture contents of cotyledons (tables 2, 3), of axes, and of entire plants at 7 days were slightly higher in −K +Na than in other treatments. Differences in moisture content between treatments at 15 days were not consistent in experiments II and III. In general, the moisture content differences were small and afforded little basis for conclusions with respect to the effect of potassium deficiency on turgidity of tissues.

CARBOHYDRATES.—Carbohydrates are of minor importance from a quantitative standpoint among the peanut seed reserves, as they constituted only 2.27% of the dry weight of the cotyledons. Data for carbohydrates in the cotyledons also show conflict between experiments II and III; this may have been because of slight differences in experimental conditions between experiments. Such differences would be accompanied by small differences in metabolic states of the developing tissues which might have been great enough to cause the conflicting results.

In experiment II the amount of carbohydrate in the cotyledons was inversely related to the amount of lipid remaining; this is what would usually be expected from a consideration of the results of PETERS (16), LASKOVSKI (10), MILLER

(14), and others with germinating fatty seeds. This was not confirmed, however, by experiment III.

In contrast to the observation of DAY (4), but in agreement with that of NIGHTINGALE *et al.* (15) on tomato stems, there was no apparent interference with the plant's ability to form starch in these experiments under −K +Na nutrient conditions, since appreciably more starch was found in the cotyledons at 7 days (experiment II) and in the histological sections of −K +Na petioles and hypocotyls at both 7 and 15 days than in the +K −Na plants.

PROTEIN.—The fate of the protein reserves was similar to that of the other complex reserves. The cotyledons behaved like the senescent leaves described by RICHARDS and TEMPLEMAN (19) in that proteolysis proceeded rapidly, thus making the amino acids available to the growing axis. The rate of protein loss from the cotyledons was increased by −K +Na conditions compared with the control (fig. 1, table 3) during the earlier period of growth; eventually the values for protein remaining in the cotyledons under the two treatments drew together again as the reserve protein approached exhaustion at the 15th day. This situation is similar to those described by RICHARDS (17) and by WALL (25), in which high respiratory rate and −K +Na nutrition were combined; in this case, however, they were not accompanied by more soluble nitrogen, at least in the cotyledons, probably because all available soluble nitrogen compounds were being used in protein synthesis in the rapidly growing axis. This would be expected, especially if potassium is necessary specifically for the early stages of nitrate reduction (15) or for the conversion of ammonium to other soluble nitrogen compounds (18), but not if potassium is necessary only for the final stages of protein synthesis from amino acids (7, 25). In this regard the writer follows the opinion expressed by TIEDJENS and WALL (24) in discussing the effect of potassium deficiency on carbohydrate formation—that these effects are "due to injury to the protoplasm because ion balance is lacking rather than to any specific unfilled catalytic role of potassium." In other words, the imbalance resulting from the substitution of potassium by sodium or any other ion disturbs protoplasmic function along a wide front, which seems plainly evident from the results of these experiments.

LIPIDS.—The curve representing the rate of loss of lipids from the cotyledons (fig. 1) parallels that for the decrease in amount of protein present, except that the rate was slightly faster over the 15-day period. In the case of lipids, as with proteins, −K +Na conditions led to more rapid loss of reserves during the earlier part of the period studied than was true under +K −Na conditions. This may have been merely a response to the greater respiratory rate of the −K +Na plants. It is thought, however, that such an explanation fails to account for the larger amount of carbohydrates in the −K +Na plants. It seems that there may be a direct effect of −K +Na nutrition to increase the lipolytic rate in the cotyledons. A hypothesis might be advanced that potassium plays a specific role in protecting the lipid reserves from the lipolytic enzymes and that, when sodium is substituted for potassium, either (*a*) the permeability to the enzymes of the membrane surrounding the lipid droplets is lowered, or (*b*) the surface per gram of lipid is so greatly increased owing to the lowering of the interfacial tension between the fats and the protoplasm that much more rapid destruction

of the fats by lipase results, or (*c*) both. Modification of the permeability of membranes surrounding an enzymatic substrate has been suggested by COVILLE (2) to account for the change of starch from a state of immunity to one of vulnerability toward diastase, or the reverse, in the continuous presence of that enzyme.

The reasoning in regard to the relationship of sodium and potassium to the protection of the lipid substrate from esterases is supported by the work of CLOWES and of HARKINS and ZOLLMAN as described by GORTNER (6). These workers showed that sodium chloride in solution greatly increased the surface of a given quantity of olive or paraffin oil dropped into the solution by lowering the interfacial tension of the system, while calcium chloride exerted the opposite effect. It will be noted that the −K −Na plants, which were grown with a slight excess of calcium in comparison with the other two treatments, retained more lipid in the cotyledons than either of the other two groups of plants both at 7 and at 15 days after planting.

Lipolysis may be so rapid under certain circumstances (experiment II) that carbohydrates originating from the breakdown are "forced" into a storage state by mass action; or, on the other hand, it may proceed somewhat more slowly (experiment III) or may be exceeded by an exalted respiratory rate to such an extent that appreciable storage carbohydrates do not result. It is thought that this is a pertinent consideration in attempting to explain the failure of experiment III to duplicate the trends in carbohydrate distribution shown by experiment II.

In explaining the occurrence of more lipid in the −K +Na cotyledons at 15 days than in the +K −Na cotyledons (the reverse of the situation at 7 days), two suggestions may be made. One is that prolonged potassium starvation resulted in malfunctioning of the translocating mechanism specifically with respect to the products of lipid destruction and that this depressed the hydrolysis of storage fats toward the end of the experimental period. There was evidence of the accumulation of carbohydrates in the cotyledons of the −K −Na plants of experiment III, which might possibly be considered as owing to interference with their translocation. Such an explanation would not only be contrary to the conclusions of NIGHTINGALE *et al.* (15), who found translocation unimpeded under −K conditions, but would also be at variance with the facts that (*a*) no anatomical differences in phloem structure between treatments was evident and that (*b*) there was no interference with amino acid translocation. Invocation of any such explanation would thus imply a translocation mechanism of great specificity, in which the transport of one class of substances normally transported could be impeded independently of other classes of substances. On the other hand, slight reduction of the cross-sectional area of phloem tissue has been reported by WATTS (26) to accompany potassium deficiency in the seedling tomato, and it does not seem impossible that a certain amount of impedance or malfunctioning of the translocation tissue could exist before it becomes evident microscopically.

The preferable explanation seems to be that the reversal of rate of utilization of lipid reserves between treatments was bound up in a manner unknown at present with the radical change in morphology and physiology of the developing seedling which intervened between the

7th and 15th days (see above, "Dry matter").

Summary

1. Virginia peanuts were grown in the dark in pots of quartz sand and were watered daily or every second day with either a complete nutrient solution (+K −Na), a solution in which sodium had been substituted for potassium (−K +Na), or one in which neither sodium nor potassium was present (−K −Na).

2. Cotyledons were harvested at the 6th or 7th and again at the 15th day after planting, were counted, weighed, and analyzed for reducing and total sugars, starch and dextrins, acid-hydrolyzable substances, soluble and insoluble nitrogen, and total lipids.

3. At 7 days carbohydrates were more abundant and lipids and crude protein less abundant in the −K +Na than in the +K −Na cotyledons. At 15 days the lipid relationship had reversed itself. Plants grown with −K −Na solution had somewhat more lipid material in their cotyledons at each stage than either of the other groups.

4. Determinations of lipolytic activity and histological study of the vascular structure of the cotyledonary petiole revealed no differences between treatments at 7 or 15 days.

5. The total dry weight of plants at both 7 and 15 days was less for −K +Na than for +K −Na plants; this is interpreted as indicating a higher respiratory rate owing to potassium deficiency.

6. The more rapid utilization of protein under −K +Na conditions is probably a reaction to the low availability of potassium for the catalysis of nitrate reduction. Since the latter condition limits the opportunity for the formation of amino acids in the −K plants, such new protein as is formed in the developing seedling axis must come from resynthesis of the proteins withdrawn from the reserve areas.

7. Histological preparations revealed the presence of several times as much starch in the seedling axis of the −K plants as in the controls. This appears to be confirmatory of the seedling's inability to utilize carbohydrate in protein synthesis.

8. No definite evidence of interference with translocation under −K conditions was found.

9. It is suggested that potassium may have a specific role in protecting the lipid substrate from lipase.

10. Additional evidence was obtained that sodium may to a certain extent alleviate potassium deficiency.

Sincere appreciation is expressed for the advice and encouragement of Professors SCOTT V. EATON and CHARLES A. SHULL, as well as other members of the Department of Botany of the University of Chicago who assisted the writer in many ways during the course of this work.

LITERATURE CITED

1. BLANCK, E. Die Bedeutung des Natriums für die Pflanze und die sogenannte Kochsalzdüngung. Fühling's Landw. Ztg. 65:441–463, 508–535. 1916.
2. COVILLE, F. V. The influence of cold in stimulating the growth of plants. Jour. Agr. Res. 20: 151–160. 1920.
3. CROOKS, D. M. Histological and regenerative studies on the flax seedling. BOT. GAZ. 95:209–239. 1933.
4. DAY, DOROTHY. Starch formation in tobacco plants deficient in potassium. Plant Physiol. 15:367–375. 1940.
5. DICKSON, J. G. The value of certain nutritive elements in the development of the oats plant. Amer. Jour. Bot. 5:301–325. 1918.

6. GORTNER, R. A. Outlines of biochemistry. 2d ed. Wiley & Sons, New York. 1938.
7. GREGORY, F. G., and SEN, P. K. Physiological studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. Ann. Bot. 1:521–561. 1937.
8. HARTWELL, B. L., and PEMBER, F. R. Sodium as a partial substitute for potassium. Rhode Island Agr. Expt. Sta. Ann. Rept. 21:243–285. 1908.
9. HOAGLAND, D. R., and ARNON, D. I. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347. 1938.
10. LASKOVSKI, N. Ueber eine chemische Vorgange bei der Keimung der Kürbissamen. Landw. Vers. Sta. 17:219–244. 1874.
11. LEHR, J. J. The importance of sodium for plant nutrition. Soil Sci. 52:237–244. 1941.
12. LONGENECKER, H. E., and HALEY, D. E. Ricinus lipase, its nature and specificity. Jour. Amer. Chem. Soc. 57:2019–2021. 1935.
13. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill Book Co., New York. 1937.
14. MILLER, E. C. A physiological study of the germination of *Helianthus annuus*. Ann. Bot. 24:693–726. 1910.
15. NIGHTINGALE, G. T.; SCHERMERHORN, L. G.; and ROBBINS, W. R. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Expt. Sta. Bull. 499. 1930.
16. PETERS, E. Zur Keimungsgeschichte des Kürbissamens. Landw. Vers. Sta. 3:1–18. 1861.
17. RICHARDS, F. J. Physiological studies in plant nutrition. III. Further studies on the effect of potash deficiency on the rate of respiration in leaves of barley. Ann. Bot. 46:367–388. 1932.
18. ———. Physiological studies in plant nutrition. XI. The effect on growth of rubidium with low potassium supply and modification of this effect by other nutrients. Part I. The effect on dry weight. Ann. Bot. 5:249–261. 1941.
19. RICHARDS, F. J., and TEMPLEMAN, W. G. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. 50:367–407. 1936.
20. SANDO, C. E. Lipides and their estimation in vegetable tissues. Plant Physiol. 3:155–184. 1928.
21 SCHIMPER, A. F. W. Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora 73:207–278. 1890.
22. SHCHERBAKOV, A. P. On the quantity and distribution of certain enzymes in the organs and tissues of pea seedlings. Biokhimiya 10:68–78. 1945.
23. SNEDECOR, G. W. Statistical methods. 3d ed. Iowa State College Press, Ames, Iowa. 1940.
24. TIEDJENS, V. A., and WALL, M. E. The importance of potassium in the growth of vegetable plants. Proc. Amer. Soc. Hort. Sci. 36:740–743. 1938.
25. WALL, M. E. The role of potassium in plants. I. Effects of varying amounts of potassium on nitrogenous, carbohydrate, and mineral metabolism of tomato plants. Soil Sci. 47:143–161. 1938.
26. WATTS, V. M. Anatomical symptoms of nitrogen, phosphorus, and potassium deficiencies in seedling hypocotyls of tomato. Arkansas Agr. Expt. Sta. Bull. 366. 1938.
27. WINTON, A. L., and WINTON, KATE B. Structure and composition of foods. I. Cereals, starch, oil seeds, nuts, oils, forage plants. Wiley & Sons, New York. 1932.

DEVELOPMENT OF THE EMBRYO OF THYMELAEA ARVENSIS

J. VENKATESWARLU

Introduction

Although the Thymelaeaceae have received considerable attention from morphologists, no accurate and detailed account of the development of the embryo of any species in the family is available. Therefore, the writer undertook an embryological study of *Thymelaea arvensis* Lamk. and published some observations elsewhere (10). Since then, he has had the benefit of certain suggestions from Dr. D. A. JOHANSEN, in the light of which it became desirable to make a revised study of the embryogeny of this species. The present paper embodies the results and includes some new illustrations. After the present investigation was completed, the writer came across the paper by SOUÈGES (9) on *Daphne mezereum*.

Material and methods

The material was collected from plants growing at Sopore, Kashmir, India, and was fixed in formalin–aceto-alcohol by Professor A. C. Joshi. After imbedding in paraffin, sections were cut 10–14 μ thick and stained in Heidenhain's iron hematoxylin.

Investigation

The first division of the zygote is transverse (fig. 1) and occurs after a few endosperm nuclei are formed. Very soon a three-celled proembryo is evident. Since the division leading to this stage was not observed, it is not possible to state definitely whether the middle cell is derived from the basal or the terminal cell of the two-celled proembryo. Fuchs (3), who studied some early stages of embryo development in *Daphne laureola*, stated that the middle cell is derived from the terminal cell of the two-celled proembryo. In *D. mezereum*, however, the middle cell is derived from the basal cell according to Souèges (9).

TABLE 1

Origin of parts of mature embryo from specific cells of proembryo

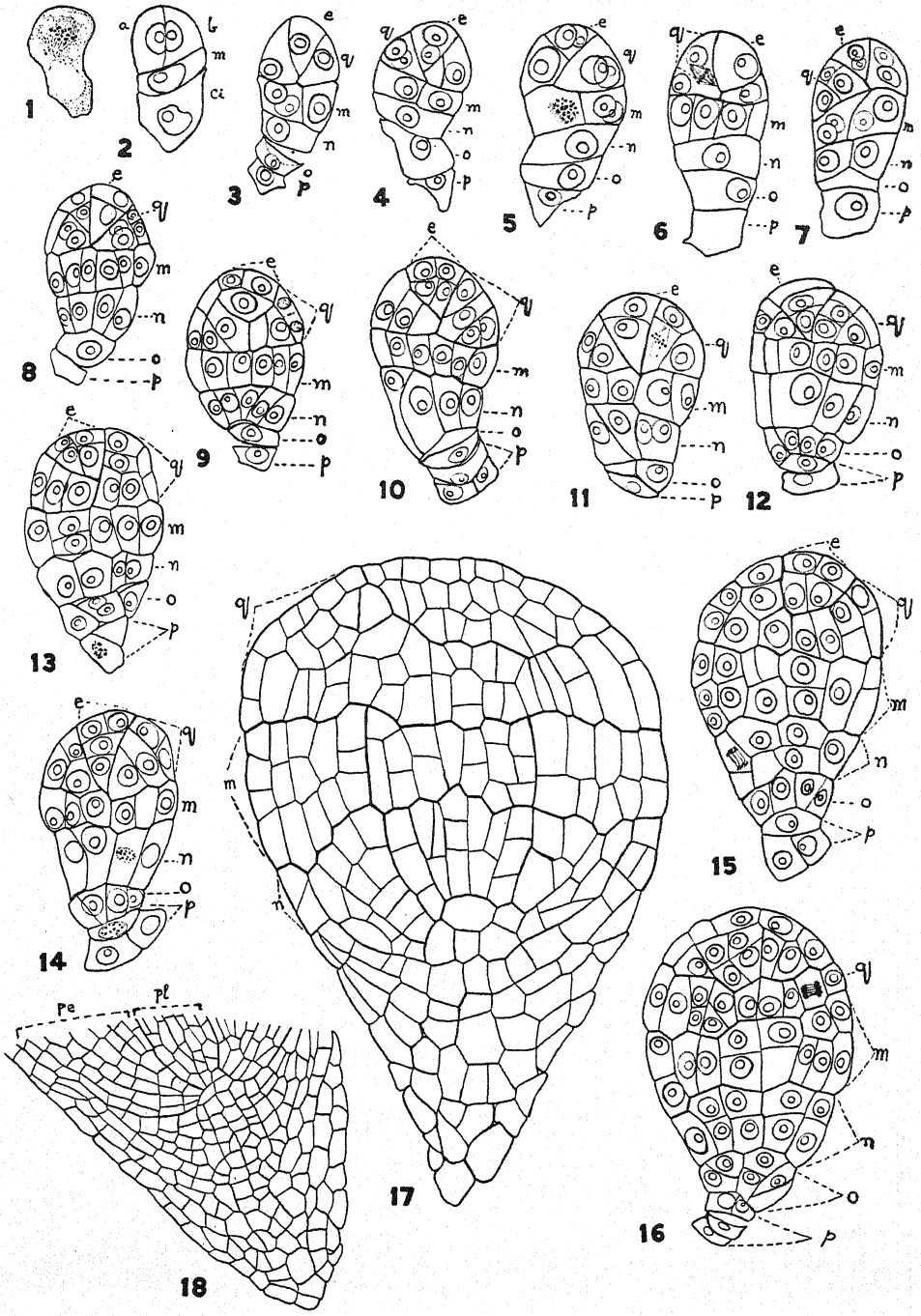
Cells of proembryo	Derivative
e	Stem apex
ct < *q*	Cotyledons
m	Hypocotyl
n	Major portion of root
o	Root apex and root cap
p	Suspensor

For convenience of description the cells of the three-celled proembryo are designated as follows: the cell nearest the micropyle, *ci;* the middle cell, *m;* and the terminal cell, *ct.*

First the terminal cell, *ct*, divides by a slightly oblique wall into two juxtaposed cells, *a* and *b*, one of them slightly smaller than the other (fig. 2). The next wall is erected in the larger of these two cells and is diagonal. The upper daughter cell arising out of this division is triangular in outline and forms the epiphyseal initial in the terminal tier. The middle cell, *m*, undergoes two longitudinal divisions in which the vertical walls are arranged perpendicularly to each other. The four cells resulting are arranged in a circumaxial manner (figs. 3, 4, 5, 7). The cell *ci* also undergoes a transverse division, producing two superposed cells, *n* and n^1. The latter of these divides transversely to form two superposed cells, *o* and *p* (figs. 3, 4, 5, 6).

The relation of the different organs of the mature embryo to specific cells of the proembryo is shown in table 1.

Segmentation in the various tiers of the proembryo proceeds in a basipetal order. The epiphyseal initial, *e*, first divides by a vertical wall (figs. 4, 5, 7). The daughter cells undergo periclinal divisions, as a result of which the epidermal initials in the region of the stem apex are differentiated. The inner cells undergo a few more divisions. The derivatives of the epiphyseal initial are perceivable as a distinct group of cells (figs. 10, 15–17), the epiphyseal region, which constitutes the stem apex. The three subepiphyseal cells, *q* (fig. 5), first divide periclinally to differentiate the epidermal initials in that region. The derivatives of the subepiphyseal cells form the cotyledons of the mature embryo (figs. 6–17). The four cells of tier *m* are first divided longitudinally (fig. 6). Periclinal divisions take place and result in the differentiation of the epidermal initials in this tier (fig. 8). The inner cells also divide longitudinally and transversely (figs. 13, 15, 16). Differentiation into the three histogenic layers occurs in this tier before the appearance of the cotyledonary pro-



FIGS. 1–18.—Stages in the development of embryo of *Thymelaea arvensis*. *a*, *b*, daughter cells formed from terminal cell (*ci*) of proembryo; *m*, middle cell of proembryo; *ci*, basal cell of three-celled proembryo; *q*, cotyledon-forming region of terminal tier; *n*, upper daughter cell formed from first division (transverse) of *ci*; *o*, *p*, upper and lower daughter cells formed from n^1 (lower daughter cell of *ci*); *pe*, periblem; *pl*, plerome. Figs. 1–17, ×100; fig. 18, ×222.

tuberances in the subepiphyseal region (fig. 17). Segmentation of the cells in the tier formed by cell *n* follows that in *m* and is similar. The derivatives of *n* (the upper daughter cell derived from the first division of cell *ci*) form the major part of the root. Cell *o* (the upper daughter cell arising from the first transverse division of n^1) divides twice longitudinally to form four circumaxially arranged cells (the hypophysis). Tangential divisions take place in these and result in the formation of an inner and an outer layer of cells (fig. 16). The inner cells undergo transverse divisions and produce the two layers of cortical initials of the root apex. The outer layers, by further periclinal and anticlinal divisions, give rise to the root cap. Cell *p* (the lower daughter cell arising out of the transverse division of n^1) divides once or twice transversely and forms a short suspensor, two or three cells in length (figs. 10, 12–16). One of the cells of the suspensor may undergo a longitudinal division (figs. 10, 14–16).

Discussion

The observations show clearly that in the embryogeny of *T. arvensis* derivatives of both the terminal and the basal cells of the two-celled proembryo contribute to the formation of the embryo proper. This is characteristic of the Asterad Type of embryo development. JOHANSEN[1] (5), for the first time, recognizes some variations under each type of embryo development. Under the Asterad Type, that of *Erodium cicutarium* described by SOUÈGES (7) is recognized as the Erodium Variation. This is characterized by the formation of an epiphysis in the terminal tier and the presence of a suspensor. *T. arvensis* is embryonomically similar to *Erodium cicutarium* and keys out to the same variation.

SOUÈGES (9) worked out in detail the development of the embryo of *D. mezereum*. In this species no epiphyseal initial is delimited, in contrast to the situation in *T. arvensis*. The basal cell (*cb*) of the two-celled proembryo divides first by a vertical or slightly oblique wall into two juxtaposed cells (*m* and *ci*). In *T. arvensis*, however, the basal cell (*ci*) and the middle cell (*m*) of the three-celled proembryo are superposed in their positions. In *D. mezereum*, *m* and *ci* divide further. The former cell segments by a vertical wall, and the latter by an oblique wall in the horizontal plane to give rise to two daughter cells termed *n* and n^1. The derivatives of *m* and *n* contribute to the formation of the hypocotyledonary region, and those of n^1 produce the root apex. No suspensor is formed. The embryogeny of *D. mezereum* thus follows the Asterad Type. However, the development of the embryo of *D. mezereum* differs from that of *T. arvensis* in the absence of an epiphysis and in the nonformation of a suspensor. Both these features are characteristic of the Penaea Variation of the Asterad Type, and the case of *D. mezereum* keys out to this variation.

In the ENGLER system of classification (2) the Thymelaeaceae, Penaeaceae, Geissolomataceae, Elaeagnaceae, and Oliniaceae are included in the subseries Thymelaeineae under the series Myrtiflorae. BENTHAM and HOOKER (1) removed the Thymelaeaceae, Penaeaceae (including the Geissolomataceae), and Elaeagnaceae to the Monochlamydeae, in which they were placed together with Laurineae and Proteaceae in the series Daphnales. HUTCHINSON (4) made a departure from both the above arrange-

[1] Dr. D. A. JOHANSEN kindly furnished relevant information relating to the variations of the Asterad Type of embryo development and about our present knowledge of the embryology of the Myrtales.

ments and erected a separate order, Thymelaeales, under which he brought together the Thymelaeaceae, Penaeaceae, Geissolomataceae, and Nyctaginaceae, the last family being included by most systematists in the order Centrospermales. The rest of the families of the Myrtiflorae (of Engler's system) are divided into two orders, the Lythrales and Myrtales. According to ENGLER, the Thymelaeales were derived from the Caryophyllales through the Lythrales.

Embryo development in the Lythraceae, Sonneratiaceae, Onagraceae, and Myrtaceae takes place according to the Onagrad Type (the outmoded Capsella Type). According to JOHANSEN (5), the embryo development in *Rhizophora mangle* "is said 'to be probably of the Capsella Type,' though the evidence is unconvincing"; in the Melastomaceae "it is probably according to the Solanad Type." In the rest of the families included under the subseries Myrtineae in the ENGLER system, our knowledge of the embryogeny is in an unsatisfactory condition. The writer has recently studied the development of the embryo of *Poivrea coccinea*, one of the Combretaceae, and found it to conform to the Erodium Variation of the Asterad Type. In the Penaeaceae and Geissolomataceae, which are included in the subseries Thymelaeineae, the embryo development keys out to the Penaea Variation, Asterad Type. In this variation the embryo is globular and no suspensor is formed.

For the Nyctaginaceae the embryo development was described in *Oxybaphus viscosus* by SOUÈGES (8) and in *Boerhaavia diffusa* and *B. repanda* by KAJALE (6). It takes place according to the Polygonum Variation, Asterad Type. In this variation a suspensor is formed, but no epiphysis is differentiated.

Considering the above facts of embryogeny in the various families with which the Thymelaeaceae have been held to be related, the present study supports a close alliance between the Thymelaeaceae and Combretaceae, on the one hand, and also with the Geissolomataceae and Penaeaceae, on the other. Further, it also supports the grouping-together of the Thymelaeaceae, Geissolomataceae, Penaeaceae, and Nyctaginaceae as proposed by HUTCHINSON, since in all these families the Asterad Type of embryo development takes place, the difference between them being only one of variation. Even within the family Thymelaeaceae two variations of the Asterad Type seem to occur. As such, the differences in variation do not seem to be of primary importance in phylogenetic considerations.

It may also be mentioned that the Nyctaginaceae differ from the other families included in the order Centrospermales in that embryo development is of the Asterad Type. Hence the embryological evidence does not favor its placement in the Centrospermales.

Summary

1. The embryo development of *Thymelaea arvensis* has been described in detail. It conforms to the Erodium Variation, Asterad Type.

2. A comparison of the embryogeny of the Thymelaeaceae with allied families supports a relationship between that family and the Combretaceae, on the one hand, and the Thymelaeaceae, Geissolomataceae, and Penaeaceae, on the other. The development of the embryo in the Nyctaginaceae differs from that in the rest of the Centrospermales and resembles that of the above-mentioned families. Consequently, HUTCHINSON's action in placing the Nyctaginaceae together with the Thymelaeaceae, Penaea-

ceae, and Geissolomataceae is favored by the embryogenic evidence.

The writer is grateful to Dr. D. A. JOHANSEN of Pomona, California, for his kind interest, critical suggestions during this investigation, and help in the preparation of the paper. He is also thankful to Professor A. C. JOSHI, Government College, Lahore, India, for the material.

ANDHRA UNIVERSITY
WALTAIR, INDIA

LITERATURE CITED

1. BENTHAM, G., and HOOKER, J. D. Genera plantarum. London. 1862–83.
2. ENGLER, A., and GILG, E. Syllabus der Pflanzenfamilien. Berlin. 1924.
3. FUCHS, A. Beiträge zur Embryologie der Thymelaeaceae. Oesterr. Bot. Zeitschr. **87**:1–41. 1938.
4. HUTCHINSON, J. Families of flowering plants, Vol. 1: Dicotyledons. London. 1926.
5. JOHANSEN, D. A. Plant embryology. (In press.)
6. KAJALE, L. B. Embryo and seed-development in the Nyctaginaeceae. I. Studies in the genus *Boerhaavia*. Jour. Indian Bot. Soc. **4**:243–254. 1938.
7. SOUÈGES, R. Embryogénie des Geraniacées. Développement de l'*Erodium cicutarium* L'Herit. Compt. Rend. Acad. Sci. (Paris) **176**:1565–1567. 1923.
8. ———. Embryogénie des Nyctaginacées. Développement de l'embryon chez l'*Oxybaphus viscosus* L'Herit. Compt. Rend. Acad. Sci. (Paris) **206**:1830–1832. 1938.
9. ———. Embryogénie des Thyméléacées. Développement de l'embryon chez le *Daphne mèzereum* L. Compt. Rend. Acad. Sci. (Paris) **214**:569–571. 1942.
10. VENKATESWARLU, J. Embryological studies in the Thymelaeaceae. I. *Thymelaea arvensis* Lamk. Jour. Indian Bot. Soc. **24**:45–56. 1945.

CURRENT LITERATURE

A Botanist in Southern Africa. By JOHN HUTCHINSON. Foreword by JOHN C. SMUTS. London: P. R. Gawthorn, Ltd., 1946. Pp. 686 + 49 pls. £2 5s.

Modern scientific writing is often impersonal in character and presentation. It is therefore a refreshing experience to read the delightful and highly informative travelogue of HUTCHINSON's expeditions in southern and central Africa, including a trip to Lake Tanganyika as guest of General SMUTS in 1930. In fact, the responsive reader will be tempted to pack his things at once and start out on his own expedition to this botanical El Dorado. Enthusiasm for the rich and extraordinary plant life of southern Africa coupled with a fine sense of humor pervades the book, as the narrative progresses from day to day and place to place, often following the tracks of early explorers. The numerous photographs, drawings, and maps add materially to the enjoyment of reading and the botanical instruction of those unable to visit Africa. HUTCHINSON's modest appraisal of his own product as a "*pot pouri* of my impressions and expressions on a botanical tour in South Africa with an account of the plants that I saw on the way" covers only part of the book but does not indicate the many observations and new ideas he managed to crowd into it.

Thus, the beginning chapters (1, 2, 8), devoted to general discussions, disclose HUTCHINSON's unreserved acceptance of WEGENER's displacement theory as the explanation of the origin and affinities of the flora of southern Africa. Some of the closing chapters (29–31) represent admirable summaries of early botanical work in South Africa, of botanical literature pertaining to its flora, and of the floral regions generally distinguished. The author's own collections are recorded at the end of each chapter and rearranged in chronological sequence in chapter 34 at the end of the book. As counted by the reviewer, descriptions and accounts of fifty-two new or inadequately known species, and new combinations as well as one new composite genus (*Zoutpansbergia*), are provided in the abundant footnotes.

Using his broad experience gained through prolonged occupation with the flora of tropical Africa and his newly acquired knowledge of the flora of southern Africa, HUTCHINSON prepared chapter 32 on "A New Phylogenetic Arrangement of the Families of Gymnosperms and Flowering Plants Represented in South Africa." This treatment supersedes in many respects his well-known two-volume set entitled *Families of Flowering Plants.* Although some "basic" groups such as Magnoliaceae, Dilleniaceae, etc., are missing in South Africa, they are indicated at least by name in brackets in order to show the entire system as now arranged by HUTCHINSON. Some significant changes concern the dissolution of the Gamopetalae and the disposition of its former members among their nearest relatives in the Archichlamydeae. The distinction of two phyla, viz., *Lignosae* and *Herbaceae* in the Dicotyledones, foreshadowed in his earlier work, is here carried to its logical conclusion, separating the "one fundamentally and remaining predominantly woody" from "the other fundamentally and remaining predominantly herbaceous." For, in the author's opinion, "the woody and herbaceous branches are considered to have been evolved side by side, the woody being probably the older." Although all stocks and their probable sources of derivation are listed in consecutive order—the pages must be read from the bottom up—the appended chart (in color) readily transmits the concepts and arrangements proposed in the text. At this point the reader is inclined to agree with HUTCHINSON's statement that "the more truly natural a system becomes the more difficult it is to express on paper." The chart also shows other changes made, viz., that the Araliales (Cornaceae and Araliaceae) are "here regarded as representing a parallelism with Umbelliferae" and that the grasses appear as the "most advanced of all" Monocotyledones.

These examples clearly indicate the great importance of this book to the botanist as a source of information and new concepts beyond the great personal pleasure awaiting him on reading it. Its usefulness could have been enhanced by the inclusion of an index to plant names, localities, and persons. The price appears reasonable in view of the excellence of its physical makeup and the fine quality of paper used.—THEODOR JUST, *Chicago Natural History Museum.*

Pflanzliche Infektionslehre: Lehrbuch der allgemeinen Pflanzenpathologie für Biologen, Förster und Pflanzenzüchter. By ERNST GÄUMANN. Basel: Verlag Birkhäuser, 1945. Pp. 611+311 figs.+90 tables.

The book under review had its origin in lectures delivered during the last 20 years by Professor GÄUMANN to his classes at the Eidgenössichen Technische Hochschule in Zürich. It is designed to acquaint the reader with the theory of plant infections, i.e., "with some biological problems which lie at the basis of the diseased state, rather than with special plant pathology" (the knowledge of individual diseases) and so "to supplement plant pathological instruction on its theoretical side."

To accomplish these ends, GÄUMANN presents the parasitological and epidemiological points of view by means of selected examples and delimits the technical terms, some of which, even now, have become part of daily usage. Since many of these terms had their origin in medicine and have experienced shifts in meaning as they entered the botanical field, deviations between phytopathological and medical thinking are indicated in the book. As a result the volume contains many comparative discussions of the theoretical aspects of infectious diseases in animals and plants. Although GÄUMANN is aware that animals and flowering plants are capable of acting as disease incitants in plants, and the book contains much interesting material bearing on the mistletoe diseases of plants, he restricts its scope to "viroses, bacterioses, and mycoses," which he designates "true infectious diseases." In spite of this limitation of its scope, and of the author's awareness of the existence of a vast domain of plant diseases in which infects play no causative role whatsoever, the book carries the subtitle "Textbook of General Plant Pathology." Also, it is surprising that, while the author states that "every disease rests primarily on a functional disturbance of the organism," he nevertheless uses the tautological expression "physiological disease" to designate the diseases not incited by infects. The book approaches a true general plant pathology in two sections of chapter 5. One of these sections deals with the morphological-anatomical manifestations of infectious diseases; the other, with their physiological manifestations. Both sections are very well done.

GÄUMANN's success in pointing out deviations between medical and phytopathological thinking reveals the inadequacy of much of the terminology and of many of the concepts current in theoretical thinking about infectious diseases. This inadequacy is especially evident in the section dealing with the responses designated "defense reactions," including the phenomena of immunization and of immunity.

GÄUMANN envisages the complicated relations between infect and host in an infectious disease as a battle and has frequent recourse to the language of conscious purposive behavior to describe and to explain the complex interplay of the activities of infect and of host. Also, in chapter 5, one reads: "An infectious disease is more than the mere reactions of the host to the infect; it forms an independent process, and independent biological complex. . . . . Incitant and host unite themselves, after infection has been established, into a life community of a higher order which is more than their mere summation."

The vast subject matter of the 571 closely printed pages of text is presented in an excellent introduction and in six chapters with the following headings: "Infection," "Infect Chains (Infektketten)," "The Parasitic Qualifications of Incitants (Die parasitische Eignung der Erreger)," "Disease Susceptibility of Hosts (Die Krankheitsbereitschaft der Wirte)," "Disease (Die Erkrankung)," and "Control (*Bekämpfung*) of Infectious Plant Diseases." There is an excellent and detailed table of contents. The literature citations are grouped in ten pages at the close of the book, and there is an index.

The book reveals an amazing mastery of the enormously diverse subject matter which is presented interestingly and effectively. There is a wealth of good graphs, photographs, drawings, tables, and maps. The volume is a contribution of the first order, and, although a textbook and not a manual, it nevertheless is a veritable mine of pertinent factual material organized on a theoretical basis.

Printing and binding are an achievement under postwar conditions. The book is an example of intellectual vigor and activity in the midst of terrific distractions. Every botanist interested in infection and infectious disease in plants will find it profitable to consult and to study this book. Finally, the book indicates that GÄUMANN's students are the beneficiaries of an unusually high level of phytopathological instruction.—GEORGE K. K. LINK.

General Horticulture. By THOMAS J. TALBERT. Philadelphia: Lea & Febiger, 1946. Pp. 452. Illus. $4.00.

This volume aims to present a "clear and concise treatise on general horticulture" for undergraduates in schools and colleges and for progressive producers. In this it succeeds in so far as north temperate deciduous fruits are concerned, but it might properly be considered as failing in its objective by one seeking full information on vegetable crops and ornamental horticulture. This situation comes about by virtue of the fact that TALBERT has used *Fruit Crops*, published in 1939, as the basis for *General Horticulture*, adding five chapters to this re-written text in order to broaden its scope. There are approximately 320 pages devoted to deciduous north temperate fruits, 20 pages to vegetable-growing, 25 pages to beautifying the home grounds, 25 pages to nut-tree culture, 30 pages to tropical and subtropical fruits, and 20 pages to handling and distributing horticultural crops.

TALBERT has succeeded in including a wide range of fundamental information, concisely stated, with selected references at the end of each chapter for those who may seek an elaboration on particular topics. He has succeeded also in tying these fundamentals to the practical problems of horticulture, drawn from his wide experience from field contacts, fruit-growers' meetings, and conferences. The result is a book with a distinctly horticultural point of view that should be found very useful in north temperate regions where the emphasis is placed upon fruits.—H. B. TUKEY.

The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat. By K. STARR CHESTER. Waltham, Mass.: Chronica Botanica Co.; New York: Stechert-Hafner, Inc., 1946. Pp. xvi+269. $5.00.

As indicated by its title, this publication deals primarily with the leaf rust of wheat, which CHESTER chooses to consider as a species, *Puccinia triticina* Erikss. The Introduction gives a brief summary of the history of the disease and the names applied to it. In following chapters the origin, distribution, and economic importance of the rust and its effect on its hosts are discussed. The importance of the leaf rust as an economic factor has only recently been understood. CHESTER points out not only that it may be very important in epiphytotic seasons, such as 1938, when a loss of more than 100,000,000 bushels of wheat occurred in the United States, but that the fairly regular general drain on the profits of agriculture may be even more important.

A summary of the information concerning the morphology, physiology, and cytology is given. The heteroecism of the rust and the role of the aecial stage are discussed. Two chapters are devoted to physiologic specialization. The reactions of the varieties of wheat differentiating the physiologic races of the rust are described. The effects of environmental factors on these reactions are discussed, and it is concluded that the reactions of the varieties Carina, Brevit, and Hussar are so unstable that accurate separation of physiologic races of the rust by their reactions is not possible. It is recommended that their use as differential varieties should be discontinued, and a reclassification of the physiologic races of the rust is given.

Two chapters are devoted to the factors affecting rust survival and development. The effect of humidity, temperature, and light upon spore germination, infection, and development of the rust through effects on its hosts are discussed. The importance of the hibernation and aestivation in the uredinial stage is emphasized. CHESTER presents evidence that there is a "critical month" in the spring, that having a mean temperature close to 50° F., which determines the intensity of the development of leaf rust. Under the heading "rust dissemination" he reviews the production of spores, wind dispersal, rate of fall, distance carried, and insect and seed carriage. Annual cycles in the development of the rust in various parts of the world and the possibility of prediction of epiphytotics are discussed. Four chapters are given to control. The factors which tend to limit the development are described, and the results which have been obtained with fungicides are summarized. Considerable space is devoted to a discussion of control through the use of rust-resistant varieties and of the methods used in breeding them.

As CHESTER has stated in the Introduction, the researches of the past quarter-century have added much to our knowledge of the nature and prevention of the cereal rusts, and there is need for a comprehensive treatment of them. Although this book is for the most part a monographic treatment of only one of the cereal rusts, they have much in common, and the volume affords a very useful reference for teachers and investigators in the fields of plant pathology and agronomy.—E. B. MAINS.

A Textbook of Systematic Botany. By DEANE B. SWINGLE. 3d ed. New York: McGraw-Hill Book Co., 1946. Pp. xv+343. $3.50.

Based on teaching experiences of 15 years and on solicited suggestions from others, the late Dean SWINGLE has synthesized and balanced the diverse elements of the oldest of botanical sciences into a pattern which is modern in approach and pedagogical in form.

A short statement on the meaning of systematic botany precedes the Introduction. The latter includes interesting facts about TORREY, GRAY, BESSEY, and COULTER and points out the usefulness of taxonomy and nomenclature in forestry, agriculture, horticulture, floriculture, range management, ecology, and paleobotany. The relation of evolution to taxonomy is presented in a most readable chapter.

Indented and parallel keys are taken from well-known floras and analyzed, and floral diagrams and floral formulas are explained and used throughout the text. Field and herbarium methods of preparing plants for further reference are discussed. Nouns and adjectives used to describe parts of plants, together with their meaning, are arranged according to plant organs.

One chapter is devoted to gymnosperms; 125 pages to the flowering plants. Fifty-seven families of dicotyledons and monocotyledons are arranged according to the system of BESSEY. Information for each family includes a general description, often a photograph or a drawing of selected plants, an emphasis on distinguishing characteristics of the plant organs, floral diagrams and formulas, and a chart for some families.

The value of the "type concept," the progress made by successive Botanical Congresses, and the principles of classification are treated from a broad and unbiased approach. The experimental method in taxonomy is discussed briefly. Historical aspects of systematic botany, which have occupied so much space in textbooks, are told in a concise and interesting manner. Useful information is given concerning the literature of systematic botany. Floras are named according to regions, and reference books for other aspects of systematics are discussed.

The present edition will have a wide appeal to those who seek a general education beyond the level of elementary botany and to the many teachers and students of systematics who will find this text adaptable to great variations in flora and curriculum.—PAUL D. VOTH.

The Genus Bazzania *in Central and South America.* By MARGARET FULFORD. "Annales Cryptogamici et Phytopathologici," Vol. 3. Waltham, Mass.: Chronica Botanica Co.; New York: G. E. Stechert & Co., 1946. Pp. xv+175. Figs. 59. $5.00.

A thorough review of even the more common leafy liverworts of the Western Hemisphere has long been a pressing need. In 1936 Miss FULFORD completed the monographic study of the species of *Bazzania* which have been collected in the United States and Canada (American Midland Naturalist 11:385–424). The collating of collections from the neighbor-nations to the south of us has resulted in the impressive monograph just published.

Following a discussion of previous arrangements of species within the genus *Bazzania*, a natural grouping under two subgenera is achieved by FULFORD. Seven species possess two-toothed leaves, while thirty-nine (one a variety) are tridentate. Of the five sections which comprise the second subgenus, only one is not represented in this hemisphere. Of the 122 species studied, 71 are reduced to synonymy. Thus much of the confusion created by STEPHANI and other early bryologists has been cleared away. *Herpetium* and *Mastigobryum* are considered synonyms of *Bazzania*. This further simplifies the taxonomy of these liverworts, thereby saving the time of the professional bryologist and lessening the frustration of the amateur.

Dr. FULFORD herself executed the excellent line drawings for each species. Leaf arrangement, denticulation of leaf margins, and thickenings of the cell wall are drawn to scale. For each species all available synonyms are cited, a complete description of the plant is given, habitats are specified, erroneous reports are discussed, collection localities are listed, and all literature references are named. The work is complete from diagnostic keys to index.

Only about twenty-one additional species or varieties were not available for study because of the unsettled conditions during the war. Many of these names will undoubtedly be adjudged synonyms.

This volume sets a high standard in research, in editing, and in publication.—PAUL D. VOTH.

GENERAL INDEX

A

Ahlgren, H. L., Variation in ecotypes of Poa pratensis 143
Alismaceae, cytological studies 262
Allium cernuum, megasporogenesis and development of embryo sac 129
Almond fruits and seeds, response to fruit thinning 550
Ammonium 2,4-dichlorophenoxyacetate, effect on Pteris longifolia 101
Antibacterial substances, in higher plants 408
Apomixis in relation to development of embryo and endosperm in Poa 531
Apples, changes during maturation 279
Asclepias, structure of bast fiber 495
Ascorbic acid, effect on apple tissue 279

B

Baeza, M. A., Response of potato plants to spray applications of certain growth-regulating substances 420
Baker, G. A., Effects of fruit thinning on almond fruits and seeds 550
Baker, Katharine C., Anatomy of Washington navel orange rind in relation to water spot 459
Bamboo, giant, affected by rust 371
Bandurski, R. S., Spectrophotometric method for determination of 2,4-dichlorophenoxyacetic acid 446
Barley, response to growth-regulating substances 510
Beal, J. M., Reactions of decapitated bean plants to certain of the substituted phenoxy compounds 166; Some results of cross-pollination on Lilium regale 526; book reviews 141, 457
Bean, histological reactions to growth-regulators 74, 166, 192; response to growth-regulators 301, 432, 510
Beeches, southern, morphology 350
Beet, sugar, response to growth-regulators 301
Benedict, H. M., Response of guayule to alternating periods of low and high moisture stresses 535
Bluegrass, Kentucky, breeding behavior and chromosome numbers 26; embryo and endosperm 531; multiple macrogametophytes 41; variation in ecotypes 143
Boatner, Charlotte H., Pigment glands of cottonseed. II. Nature and properties of gland walls 484
Bonner, James, The role of organic matter, especially manure, in the nutrition of rice 267
Borthwick, H. A., Action spectrum for the photoperiodic control of floral initiation of short-day plants 1
Brooks, R. M., Effects of fruit thinning of almond fruits and seeds 550
Brown, W. V., Cytological studies in the Alismaceae 262
Buchholz, J. T., Volumetric studies of seeds, endosperms, and embryos in Pinus ponderosa during embryonic differentiation 232; book review 299
Buckwheat, response to growth-regulators 301
Buffer properties of citrus peels 398
Burris, R. H., Characteristics of the nitrogen-fixing enzyme system in Nostoc muscorum 254

C

Calcium carbonate, relation to persistence of 2,4-dichlorophenoxyacetic acid in soil 510
Caplin, S. M., Growth and morphology of tobacco tissue cultures in vitro 379
Carbohydrates, water-soluble, in guayule 295
Cardiocarpon from Iowa coal fields 51
Castillon, Leah E., Pigment glands of cottonseed. II. Nature and properties of gland walls 484
Castor bean, effects of photoperiod, light intensity, and grafting on floral initiation 556
Cellulose fibers, structure 495
Chester, K. S., "The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat" 589
Chromosome numbers, Alismaceae 262; Poa pratensis 26
Citrus leaf stomata: structure, composition, and pore size 476
Citrus peels, organic acids and buffer properties 308
Citrus rind, anatomy 459
Clover, red, response to growth-regulators 301
Cocklebur, photoperiodic control of floral initiation 1
Corn, response to growth-regulators 301, 432
Cotton fiber, structure 495
Cottonseed, pigment glands 484
Cytological studies, Alismaceae 262; Poa pratensis 26; Uromyces aloës 245

D

Darlington, C. D., "Chromosome atlas of cultivated plants" 141
Dasturella divina, morphology of spore forms and heteroecism 371
Dendrocalamus strictus, affected by rust 371
Dichlorophenoxyacetate, ammonium 2,4-, effect on Pteris longifolia 101
Dichlorophenoxyacetate, sodium 2,4-, effect on potato plants 420
Dichlorophenoxyacetic acid (2,4), applied with fertilizer 414; effect on field crops in treated soil 432; effect on flower formation in pineapple 64; effect on growth of grass 414; herbicidal properties 301, 414; persistence in soil 510; removal from soil by leaching 186; as a spray-oil amendment 286; spectrophotometric method for determination 446
Domingo, W. E., Effect of duration and intensity of light upon flowering in several varieties and hybrids of castor bean 556
Dyer, H. J., Influence of potassium and sodium on metabolism of peanut cotyledons during germination 570

E

Ebeling, Walter, Preliminary results with the use of 2,4-dichlorophenoxyacetic acid as a spray-oil amendment 286
Ecotypes of Poa pratensis 143
Eggers, Virginia, The effect of indoleacetic acid upon initiation and development of hypocotyledonary bud primordia in flax 114
Ellison, J. H., Response of potato plants to spray applications of certain growth-regulating substances 420
Embryo development, in Poa pratensis 531; in Thymelaea arvensis 581
Embryo sac, in Allium cernuum 129; in pomegranate 394
Embryology of Lilium regale 526
Embryonic differentiation in pine seeds 232
Endosperm development in Poa pratensis 531
Eny, D. M., Ether-soluble organic acids and buffer properties of citrus peels 398

F

Fagaceae, morphology of Nothofagus 350
Fibers, cellulose, structure 495
Flax, reaction to growth-regulating substances 114
Floral initiation, action spectrum for photoperiodic control 1; in castor bean, affected by photoperiod, light intensity, and grafting 556; control in pineapple 64; in Mangifera indica 136
Florin, Rudolf, "Die Koniferen des Oberkarbons und des unteren Perms" 299
Fulford, Margaret, "The genus Bazzania in Central and South America" 590

G

Gäumann, Ernst, "Pflanzliche Infektionslehre: Lehrbuch der allgemeinen Pflanzenpathologie für Biologen, Förster und Pflanzenzüchter" 587
Gopalakrishnan, K. S., Morphology of spore forms and heteroecism of the giant bamboo rust, Dasturella divina 371
Griffin, Jean H., Changes which occur in apple tissue upon treatment with various agents and their relation to the natural mechanism of softening during maturation 279
Growth-curves, of tobacco tissue cultures 379
Growth-regulating substances, applied with fertilizer 414; effect on flower formation in pineapple 64; effect on grass 414; effect on hypocotyledonary buds in flax 114; effect on potato plants 420; effect on Pteris longifolia 101; effect on tree seedlings 219; as herbicides 301, 414, 432; histological responses of bean 74, 166, 192; persistence in soil 186, 510; soil treatment 432; in spray oil 286
Guayule, response to alternating periods of low and high moisture stresses 535; water-soluble carbohydrates 295

H

Hall, Catherine M., Pigment glands of cottonseed. II. Nature and properties of gland walls 484
Hamner, K. C., book review 458
Hanks, R. W., Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching 186
Hayes, Lesley E., Survey of higher plants for presence of antibacterial substances 408
Hendricks, S. B., Action spectrum for the photoperiodic control of floral initiation of short-day plants 1
Herbicidal properties of 2,4-dichlorophenoxyacetic acid 301, 414, 432
Histological responses to plant growth-regulators, in bean 74, 166, 192; in Pteris longifolia 101
Histological responses to wounding, in bean 192
Histology, of orange rind 459; of tobacco tissue cultures 379
Howard, W. L., "Luther Burbank: a victim of hero worship" 457
Hutchinson, J., "A botanist in southern Africa" 587
Hydrogen peroxide, effect on apple tissue 279
Hypocotyledonary buds in flax 114

I

Indoleacetic acid, effect on hypocotyledonary buds in flax 114; effect on potato plants 421; histological responses in bean 74
Inulin, analysis in guayule 295
Isopropyl-N-phenyl carbamate, effect on field crops in treated soil 432

J

Janaki Ammal, E. K., "Chromosome atlas of cultivated plants" 141
Just, Theodor, book review 587

K

Kertesz, Z. I., Changes which occur in apple tissue upon treatment with various agents and their relation to the natural mechanism of softening during maturation 279
King, J. R., Development of ovule and megagametophyte in pomegranate 394
Kraus, E. J., Growth-regulating substances as herbicides 301; book review 457
Kries, Olive H., Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime 510

L

Langdon, LaDema M., The comparative morphology of the Fagaceae. I. The genus Nothofagus 350
Levulins, analysis in guayule 295
Light intensity, effect on castor bean 556
Lilium regale, cross-pollination 526
Link, G. K. K., The effect of indoleacetic acid upon initiation and development of hypocotyledonary bud primordia in flax 114; book review 587
Lynch, S. J., Flower-bud formation and development in Mangifera indica 136

M

MacNeil, Alan and Esther, "Garden lilies" 457
McRary, W. L., Response of guayule to alternating periods of low and high moisture stresses 535
Macrogametophytes in Poa pratensis 41
Mains, E. B., book review 589
Maki, T. E., Effects of pre-storage treatment of hardwood and pine seedlings with α-naphthaleneacetic acid 219

Mangifera indica, floral initiation 136
Manure, effect on rice seedlings 267
Marigold, response to growth-regulating substances 510
Marth, P. C., Selective herbicidal effects of 2,4-dichlorophenoxyacetic acid applied to turf in dry mixtures with fertilizer 414
Megagametophyte, in pomegranate 394
Megasporogenesis, in Allium cernuum 129; in Vanilla 449
Metabolism of peanut cotyledons 570
Microsporogenesis in Vanilla 449
Milkweed bast fiber, structure 495
Mitchell, J. W., Growth-regulating substances as herbicides 301; Selective herbicidal effects of 2,4-dichlorophenoxyacetic acid applied to turf in dry mixtures with fertilizer 414
Monosaccharides, analysis in guayule 295
Murphy, J. B., Megasporogenesis and development of the embryo sac of Allium cernuum 129
Murray, Mary A., A comparison of histological responses of bean plants to tryptophane and to low concentrations of indoleacetic acid 74; Histological responses of bean plants to nicotine and to wounding 192
Mustard, Margaret J., Flower-bud formation and development in Mangifera indica 136
Mustard, response to growth-regulating substances 432, 510

N

Naphthaleneacetic acid, effect on flower formation in pineapple 64; effect on potato plants 421; effect on tree seedlings 219
Narasimhan, M. J., Morphology of spore forms and heteroecism of the giant bamboo rust, Dasturella divina 371
Nicotine, histological responses in bean 192
Nielsen, E. L., Breeding behavior and chromosome numbers in progenies from twin and triplet plants of Poa pratensis 26; Developmental sequence of embryo and endosperm in apomictic and sexual forms of Poa pratensis 531; The origin of multiple macrogametophytes in Poa pratensis 41; Variation in ecotypes of Poa pratensis 143
Nitrogen-fixation by Nostoc muscorum 254
Nitrogen nutrients, effect on rice seedlings 267
Nostoc muscorum, nitrogen-fixation 254
Nothofagus, morphology 350
Nutrition of rice seedlings 267

O

Oats, response to growth-regulators 301
Orange rind, anatomy in relation to water spot 459
Orange tree, effect of 2,4-dichlorophenoxyacetic acid in spray oil 286
Organic acids in citrus peels 308
Ovule in pomegranate 394

P

Parker, M. W., Action spectrum for the photoperiodic control of floral initiation of short-day plants 1
Peanut cotyledons, metabolism 570
Pectic transformations in apples 279
Phenoxy compounds, histological effect on bean 166
Photoperiod, action spectrum in control of floral initiation 1; effect on castor bean 556; effect on pineapple 64
Pigment glands of cottonseed 484
Pine seedlings, effects of naphthaleneacetic acid 219
Pine seeds, embryonic differentiation 232
Pineapple, control of flower formation and fruit size 64
Poa pratensis, breeding behavior and chromosome numbers 26; development of embryo and endosperm in apomictic and sexual forms 531; multiple macrogametophytes 41; variation in ecotypes 143
Pollination, cross, effect in Lilium regale 526
Pomegranate, ovule and megagametophyte 394
Potassium, effect on metabolism of peanut cotyledons during germination 570
Potato plants, reaction to growth-regulating substances 420
Pringsheim, E. G., "Pure cultures of algae: their preparation and maintenance" 141
Pteris longifolia, responses to ammonium 2,4-dichlorophenoxyacetate 101
Punica, megagametophyte 394

Q

Quercus seedlings, effects of naphthaleneacetic acid 219

R

Radish, response to growth-regulators 301
Randia dumetorum, affected by rust 371
Rape, response to growth-regulators 301
Reed, Fredda D., On Cardiocarpon and some associated plant fragments from Iowa coal fields 51
Rice, nitrogen nutrition 267; response to growth-regulators 301
Ricinus, floral initiation 556
Robinia seedlings, effects of naphthaleneacetic acid 219
Rollins, Mary L., Photomicrographic comparison of milkweed bast and cotton lint in relation to cell-wall structure of other natural cellulose fibers 495; Pigment glands of cottonseed. II. Nature and properties of gland walls 484
Rubber content of guayule, affected by soil moisture stresses 535
Rust, on Aloe 245; on giant bamboo and on Randia dumetorum 371

S

Sagittaria, chromosome numbers 262
Scott, Flora M., Anatomy of Washington navel orange rind in relation to water spot 459
Scully, N. J., Action spectrum for the photoperiodic control of floral initiation of short-day plants 1; Effect of duration and intensity of light upon flowering in several varieties and hybrids of castor bean 556
Seeds, Carboniferous 51; embryonic differentiation in pine 232
Sinclair, W. B., Ether-soluble organic acids and buffer properties of citrus peels 398
Slattery, M. C., Analysis of levulins, inulin, and monosaccharides in guayule 295; Response of guayule to alternating periods of low and high moisture stresses 535

Smith, D. C., Variation in ecotypes of Poa pratensis 143
Smith, G. M., book review 141
Smith, Ora, Response of potato plants to spray applications of certain growth-regulating substances 420
Sodium, effect on metabolism of peanut cotyledons during germination 570
Soil moisture, effect on guayule 535
Soil treated with growth-regulating substances 186, 432, 510
Soybean, photoperiodic control of floral initiation 1
Spectrophotometric method for determination of 2,4-dichlorophenoxyacetic acid 446
Spray-oil amended with 2,4-dichlorophenoxyacetic acid 286
Stewart, W. S., Preliminary results with the use of 2,4-dichlorophenoxyacetic acid as a spray-oil amendment 286
Stoeckeler, J. H., book review 458
Stomata in citrus leaf, structure, composition, and pore size 476; in citrus rind 459
Strickler, B., Responses of Pteris longifolia to applications of ammonium 2,4-dichlorophenoxyacetate 101
Swamy, B. G. L., On the life-history of Vanilla planifolia 449
Swingle, D. B., "A textbook of systematic botany" 589

T

Talbert, T. J., "General horticulture" 588
Taylor, D. L., Growth of field crops in soil treated with chemical growth-regulators 432
Thinning, fruit, effect on almond fruits and seeds 550
Thirumalachar, M. J., A cytological study of Uromyces aloës 245; Morphology of spore forms and heteroecism of the giant bamboo rust, Dasturella divina 371
Thymelaea arvensis, development of embryo 581
Tobacco tissue cultures 379
Traub, H. P., Analysis of levulins, inulin, and monosaccharides in guayule 295
Trees, seedlings affected by plant growth-regulators 219
Tryptophane, histological responses in bean 74
Tukey, H. B., book review 588
Turrell, F. M., Citrus leaf stomata: structure, composition, and pore size in relation to penetration of liquids 476

U

Uromyces aloës, cytological study 245

V

Van Overbeek, J., Control of flower formation and fruit size in the pineapple 64
Vanilla planifolia, life-history 449
Venkateswarlu, J., Development of the embryo of Thymelaea arvensis 581
Voth, P. D., book reviews 589, 590

W

Water spot on orange 459
Way, R. D., Effects of pre-storage treatment of hardwood and pine seedlings with α-naphthaleneacetic acid 219
Wheat, response to growth-regulators 301, 432
Whiting, A. Geraldine, A comparison of histological responses of bean plants to tryptophane and to low concentrations of indoleacetic acid 74; Histological responses of bean plants to nicotine and to wounding 192
Whyte, R. O., "Crop production and environment" 458
Wilde, S. A., "Forest soils and forest growth" 458
Wilson, P. W., Characteristics of the nitrogen-fixing enzyme system in Nostoc muscorum 254
Wounding, histological responses in bean 192

X

Xanthium saccharatum, photoperiodic control of floral initiation 1